TABLE OF CONTENTS

PREFACE

The National Center for Toxicological Research (NCTR), one of the six U.S. Food and Drug Administration (FDA) product centers and the Office of Regulatory Affairs (ORA), is dedicated to the conduct of fundamental and applied research to provide FDA with a stronger scientific base for making regulatory decisions. NCTR, a component of the Jefferson Laboratories of the FDA in Jefferson, Arkansas, is located approximately 30 miles south of Little Rock.

The mission of NCTR is to conduct peer-reviewed scientific research that supports the FDA's current and anticipated future regulatory needs. This involves fundamental and applied research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by the FDA and the development of improved methods for assessment of human exposure, susceptibility and risk. In addition to its FDA support, NCTR leverages its resources by conducting integrated research programs with other FDA centers and the ORA and through collaborative agreements with other government agencies, academia, and industry. NCTR receives guidance and advice on the relevance and quality of its research programs from an extramural Science Advisory Board, its liaison members from each of the other FDA centers and ORA, and other stakeholders.

The NCTR views its FDA role as a principal in development and modification of toxicology safety standards. New health concerns, such as BSE, AIDS, pediatric initiatives, skin cancer, antibiotic resistance, and emerging foodborne pathogens, in addition to traditional concerns, are challenging the conventional ways in which the regulatory agencies (both national and international) set safety standards designed to protect public health. Examples of how NCTR is supporting and meeting standard-setting challenges of the FDA include:

- Developing transgenic animals and cells to better predict the risk to humans of products regulated by the FDA.
- Working with all of the FDA product centers to better predict human risk by developing new statistical models of risk for microbial contamination and conducting studies to assess the risk from mixtures of hazardous products.
- Modifying the standard animal bioassay to better assess the carcinogenic risk of FDA-regulated products to humans. Chronic studies on chloral hydrate, a pediatric sedative, have been completed and are being considered for use in standard setting.
- Using an operant test battery (developed at NCTR) to measure behavioral and neurological changes following drug exposure across species (rodents, non-human primates, and humans), and at various stages of development (children).
- Developing quick and accurate methods of measuring foodborne contamination, and antibiotic resistance.
- Developing and validating methods to detect seafood decomposition (Fresh Tag™) and for characterizing antibiotic-resistant bacteria using mass spectrometry.
- Developing a phototoxicology facility to study how sunlight and chemicals work together to cause damage to skin.

Other important areas of research supported in part by outside funding included effects of anticonvulsants on complex brain functions in non-human primates, antibiotic resistance associated with competitive exclusion products, and development of risk assessment tools to better extrapolate animal toxicity data to humans. Perhaps of greater importance to our research accomplishments was the benefit gained by sharing knowledge through collaborations with scientific staff of other government, academic, and industrial institutions. I am proud to present this report that summarizes these and other NCTR research accomplishments and plans for the fiscal years 2000-2001.

Daniel A. Casciano, Ph.D.
Director, NCTR

SCIENCE ADVISORY BOARD

Function

One of the keys to maintaining a high quality research organization is the utilization of an outside body of experts, such as a Science Advisory Board (SAB), to periodically review the quality as well as the direction of the research. The NCTR SAB advises the Director in establishing, implementing and evaluating the research programs that assist the Commissioner of the Food and Drug Administration (FDA) in fulfilling regulatory responsibilities. This additional review ensures that the research programs at NCTR are scientifically sound and pertinent to the FDA.

FY2000 Accomplishments

At its June 2000 meeting, the Board reviewed and approved the site visit reports of the Center's Division of Microbiology and Estrogen Disruptor Knowledge Base project.

The board also received updates on their previous recommendations from the site visits of the review of the Center's programs on Biochemical Toxicology, Genetic Toxicology and Molecular Epidemiology. These updates are summarized in the minutes of the SAB.

The site visit reports and the minutes of the SAB meetings may be obtained by contacting the NCTR Office for Washington Operations, 5600 Fishers Lane, HFT-10, Rockville, MD 20857, E-mail: bjewell@nctr.fda.gov. or telephone: 301-827-6696. *(FDA employees may access the SAB site at http://www.intranet.nctr.fda.gov/sab/).*

In 2000, the Board lost by retirement, Drs. Marion Anders, University of Rochester; Robert Anderson, West Virginia School of Environmental Education Inc.; Marcy Rosenkrantz, Air Force Rome, NY Laboratory; and Charles Wilkins, University of Arkansas. Currently, recruitment is underway to fill these four vacancies as the Board begins its 27th year of service to the FDA and the Center.

Membership Roster**

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Daniel Acosta, Jr. Dean, School of Pharmacy	University of Cincinnati	6/30/03	Pharmacology and Toxicology
Dr. Catherine W. Donnelly Associate Dean, College of Agriculture & Life Sciences	University of Vermont Burlington, VT	6/30/02	Microbiology/Food Science
Dr. Nancy Ann Gillett Sr. Vice President Sierra Biomedical	Charles River Laboratories	6/30/03	Veterinary Medicine and Pathology
Dr. Stephen S. Hecht Wallin Land Grant Professor of Cancer Prevention	University of Minnesota Cancer Center Minneapolis, MN	06/30/02	Chemistry
Dr. Cecil Pickett Executive Vice President Discovery Research	Schering-Plough Research Institute	06/30/03	Cell Biology and Biochemistry
Dr. Leonard M. Schechtman Executive Secretary Associate Deputy Director for Washington Operations, NCTR	FDA/NCTR Rockville, MD	Ongoing	Research Administration

** Four new members are currently being processed for appointment to the Board

BIOCHEMICAL TOXICOLOGY

Director: ***Frederick A. Beland, Ph.D.***
Telephone: **870-543-7205**
Toll Free: **800-638-3321**
E-mail: **fbeland@nctr.fda.gov**

Lonnie Blankenship conducts the ^{32}P-postlabeling assay to examine DNA adducts.

Executive Summary

The Division of Biochemical Toxicology conducts fundamental and applied research specifically designed to define the biological mechanisms of action underlying the toxicity of products either regulated by or of interest to the Food and Drug Administration (FDA). This research centers on assessing the toxicities and carcinogenic risks associated with specific chemicals and gene-nutrient interactions, and the introduction of new techniques to assess toxicities and carcinogenic risks. The risk assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent carcinogenic risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, cellular and molecular biology, immunology, nutritional biochemistry, and pharmacology. It is supported by sound technical skills, the availability of state-of-the-art equipment, and internal and external collaborations and funding.

A major emphasis within the Division is to conduct research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences, National Toxicology Program (NIEHS/NTP). This focus reflects the fact that the NCTR has superb animal facilities supported by a staff of scientists with strong multidisciplined mechanistic research experience; as such, the Center has the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address the FDA's needs. While acknowledging the limitations of animal bioassays, these studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

The Division's initial investigations for the NIEHS/NTP included fumonisin B_1, a mycotoxin found in corn, which was nominated by the FDA Center for Food Safety and Applied Nutrition (CFSAN); chloral hydrate, a pediatric sedative, in response to a request made by the Center for Drug Evaluation and Research (CDER); malachite green, a therapeutic agent used in aquaculture that was nominated by the Center for Veterinary Medicine (CVM); urethane, a contaminant of certain food products, at the request of

CFSAN to assist this center to establish regulatory levels for this carcinogen; and riddelliine, a pyrrolizidine alkaloid present in various herbal preparations. More recently the scope of the NIEHS/NTP investigations has been expanded to include a series of endocrine-active compounds, including genistein, ethinyl estradiol, nonylphenol, methoxychlor, and vinclozolin. In addition, a major effort has started in the area of phototoxicity, with emphasis on the potential interaction between ultraviolet (UV) light and substances found in over-the-counter cosmetics. Initial studies have focused on alpha and beta-hydroxy acids and during the coming year, the scope will be expanded to include *Aloe vera* and retinyl palmitate. Studies will also be initiated to include toxicological evaluations on antiretroviral drugs, including zidovudine, lamivudine, nevirapine, and nelfinavir.

Traditional chronic carcinogenicity bioassays are both very expensive and lengthy; thus, the development of alternative methods of assessing carcinogenic potential should be of great value. One approach that is currently being investigated is the neonatal mouse tumorigenicity assay. The advantages of this method are that only limited amounts of test material are required, a direct assessment is obtained as to whether or not the agent acts through a genotoxic mechanism, and less time is required to elicit a carcinogenic response. This alternative bioassay has been applied to benzodiazepines, antihistamines, lipid peroxidation products, estrogens, antiestrogens, peroxisome proliferators, lipid peroxidation inducers, proton pump inhibitors, mycotoxins, and a series of known human carcinogens.

An ongoing goal within the Division is to exploit both the immunogenicity and the antigenicity of toxicants, metabolites, and DNA adducts to develop and apply immunochemical methods combined with mass spectral techniques to address problems of regulatory concern. This technology has been applied to fumonisin B_1, fumonisin B_2, and fumonisin B_3, aromatic amine DNA adducts, nucleoside analogues of anti-HIV drugs, and etheno-type DNA adducts formed by urethane. More recently, studies were initiated to prepare antibodies against ultraviolet (UV) photoproducts in support of the Division's phototoxicity effort. In addition, Division investigators have developed methodologies to assay catechol formation from endogenous estrogens.

A major focus of the Division is to develop analytical methodology based on mass spectrometry to measure biomarkers of exposure and toxicity in animals and humans in conjunction with studies that define mechanisms of toxicity. Toward this end, liquid chromatography-mass spectrometry (LC/MS) methods were developed and applied to analyze genistein and daidzein, compounds found in soy products. Additional LC/MS methods are being developed for the sensitive and selective detection of DNA adducts formed through metabolic activation of exogenous chemical carcinogens and by reactive oxygen species that are byproducts of normal aerobic metabolism.

A strong emphasis within the Division has been in the area of nutritional folic acid deficiency. As part of this program, Division investigators have evaluated the progression of global DNA hypomethylation and promoter region hypermethylation in the tumor suppressor gene, p53. They have also assessed thiol metabolites associated

with folate-dependent homocysteine metabolism and have shown that increased plasma homocysteine, a risk factor for cardiovascular disease and certain birth defects, is associated with a parallel increase in S-adenosylhomocysteine. In further work they demonstrated that abnormal folate metabolism was associated with polymorphisms in the methylene tetrahydrofolate reductase and methionine synthase reductase genes in mothers of children with Down Syndrome.

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Beland, Frederick

- ***Tumorigenicity of Chloral Hydrate in $B_6C_3F_1$ Mice*** — ***E0211601*** — ***CDER*** — ***Agent-Driven***

Objective(s):

To determine the effect of animal age and duration of exposure upon the tumorigenicity of chloral hydrate in female $B_6C_3F_1$ mice.

FY 2000 Accomplishments:

Tumor study completed and defended before NTP.

FY 2001 Plans:

Modify final report based upon recommendation of NTP review committee.

- ***Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in $B_6C_3F_1$ Mice*** — ***E0212001*** — ***CFSAN*** — ***Agent-Driven***

Objective(s):

To determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in $B_6C_3F_1$ mice.

FY 2000 Accomplishments:

1) Draft pathology report completed.
2) Metabolism/adduct study continued.
3) Two papers on mass spectrometry methods for the detection of etheno DNA adducts submitted.

FY 2001 Plans:

1) Prepare draft NTP final report.
2) Complete mechanistic studies associated with bioassay.
3) Initiate oncogene analyses.

- ***DNA Adducts of Tamoxifen*** — ***E0701101*** — ***None*** — ***Agent-Driven***

Objective(s):

The nonsteroidal antiestrogen tamoxifen, which is currently being used in clinical trials as a chemoprotective agent against breast cancer, has been associated with the induction of certain malignancies. In order to determine if tamoxifen is acting through a genotoxic mechanism, this project will characterize DNA adducts from suspected tamoxifen metabolites and develop methods for their detection and quantitation.

FY 2000 Accomplishments:

1) Synthesis completed on desmethyl tamoxifen, didesmethyltamoxifen, and their α-hydroxy derivatives.
2) Rats treated with various tamoxifen derivatives.

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

3) Characterization of the major DNA adduct formed by α-hydroxy-*N*-desmethyl-tamoxifen *in vitro* and *in vivo* published.
4) DNA adduct formation and mutant induction in Sprague-Dawley rats treated with tamoxifen and its derivatives presented at American Association for Cancer Research (AACR) meeting.
5) Guest Worker continued experiments for Ph.D. thesis.
6) Initiated experiments with Premarin.

FY 2001 Plans:

1) Complete Big Blue Rat mutagenesis work with tamoxifen and derivatives.
2) Continue Premarin work, with emphasis on DNA adducts from equilenin quinone.
3) Initiate DNA adduct studies in women receiving tamoxifen.

PI: Boudreau, Mary

◆ ***Toxicity of Aloe Vera Photo Study*** — ***X10017*** — ***None*** — ***Agent-Driven***

Objective(s):

Aloe vera is known to contain substances capable of promoting cell proliferation and altering cellular metabolism, conditions recognized as cancer promoting activities. Furthermore, studies have demonstrated that some components of *Aloe vera* when applied to the skin of mice in conjunction with UV radiation induced skin tumors, suggesting that *Aloe vera* in sun-skin-care preparations may synergize with UV radiation to induce skin cancer. The objective of the *Aloe vera* photo study is to determine whether topical application of *Aloe vera* results in increased sensitivity to simulated solar light, enhanced epithelial cell proliferation, and increased DNA damage from UV radiation.

FY 2000 Accomplishments:

Not Applicable.

FY 2001 Plans:

1) Conduct literature review.
2) Prepare protocol.
3) Initiate studies.

PI: Chou, Ming

◆ ***A Study of Genotoxic and Secondary Mechanisms of Riddelliine Carcinogenesis*** — ***E0213301*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To study the mechanisms of direct-acting genotoxicity (involving exogenous DNA adduct formation) of riddelliine.

2) To analyze riddelliine-derived DNA adducts in target tissues of rats treated with riddelliine as part of the NTP chronic study, and from male and female rats to be treated at the NCTR for a shorter period of time with riddelliine and its reactive metabolite, dehydroriddelliine.
3) If a dehydroretronecine-modified DNA adduct is detected in the liver tissues of animals treated with riddelliine, propose to determine whether or not this DNA adduct is also formed in animals treated with other tumorigenic pyrrolizidine alkaloids.
4) To compare the metabolic activation pathways and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine, retrorsine, and monocrotaline, and a non-tumorigenic pyrrolizidine alkaloid, retronecine in rat and human liver microsomal systems.

FY 2000 Accomplishments:

1) A ^{32}P-postlabeling/HPLC method was developed for the identification and quantification of dehydroretronecine (DHR) DNA adducts.
2) Synthesized dehydroretronecine and dehydroretronecine-modified dG-3'-phosphate adducts.
3) Using this methodology, eight DHR-derived DNA adducts were detected in the livers of female rats treated with riddelliine.
4) Two of the adducts were identified.
5) Two manuscripts have been submitted.

FY 2001 Plans:

1) Continue characterization of dehydroretronecine DNA adducts.
2) Compare and quantify riddelliine-derived adduct levels in target and non-target tissues of F344 rats and $B_6C_3F_1$ mice.
3) Conduct human microsomal metabolism studies with riddelliine.
4) Determine the role of riddelliine *N*-oxide in riddelliine carcinogenesis.

◆ ***Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B_1(AFB_1)-Induced Carcinogenesis on Male F344 Rats Fed Methyl-Deficient Diets*** — ***E0695201*** — ***None*** — ***Concept-Driven***

Objective(s):

To study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB_1-induced carcinogenesis in male F344 rats. The results of these studies will:

1) Test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB_1-induced carcinogenesis.

2) Evaluate the correlations between the effects of DR and MD on the formation of AFB_1-induced preneoplastic foci and tumors and various biomarkers during the post-initiation stages of carcinogenesis.

FY 2000 Accomplishments:

A manuscript describing relationships between methyl deficiency, dietary restriction, hepatic cell proliferation, and telomerase activity was published.

FY 2001 Plans:

Assay oxidative damage in liver DNA from rats fed methyl-deficient diets and determine whether or not oxidative damage can be reduced by dietary restriction.

PI: Culp, Sandra

◆ ***Two-Year Bioassay in Mice Administered Malachite Green or Leucomalachite Green in the Diet*** ***E0212701*** ***CVM*** ***Agent-Driven***

Objective(s):

To determine the risk associated with exposure to malachite green or leucomalachite green.

FY 2000 Accomplishments:

1) Bioassay and mechanistic studies are ongoing.
2) A paper describing mechanistic studies has been accepted. Another paper was published.

FY 2001 Plans:

1) Complete In-life phase of bioassay with malachite green/leucomalachite green in mice.
2) Complete pathology on bioassay (NTP report will be completed in 2002).
3) Mechanistic studies will continue with emphasis on the role of demethylated metabolites in the metabolic activation of malachite green/leucomalachite green.

◆ ***Two-year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet*** ***E0212801*** ***CVM*** ***Agent-Driven***

Objective(s):

To determine the risk associated with exposure to malachite green or leucomalachite green.

FY 2000 Accomplishments:

1) Bioassay and mechanistic studies are ongoing.
2) A paper describing mechanistic studies has been accepted. Another paper was published.
3) An addenum was initiated to investigate the mutagenicity of malachite green and derivatives in the Big Blue Rat.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) Complete In-life phase of bioassay with malachite green/leucomalachite green in rats.
2) Complete pathology on bioassay (NTP report will be completed in 2002).
3) Mechanistic studies will continue, with emphasis on the role of demethylated metabolites in the metabolic activation of malachite green/leucomalachite green.
4) Initiate In-life phase of Big Blue Rat experiment with malachite green/ leucomalachite green.

◆ ***Phototoxicity of Retinyl Palmitate*** ***X10021*** ***CFSAN*** ***Agent-Driven***

Objective(s):

To evaluate the photocarcinogenicity of retinyl palmitate and conduct mechanistic studies to determine the relevance of the results obtained in the selected animal model.

FY 2000 Accomplishments:

Not Applicable.

FY 2001 Plans:

1) Conduct literature review.
2) Prepare protocol.
3) Initiate mechanistic studies.

PI: Dalu, Abraham

◆ ***The Effects of Dietary Genistein on the Growth of Chemically-Induced Mammary Tumors in Ovariectomized and Intact Rats*** ***E0702701*** ***None*** ***Agent-Driven***

Objective(s):

To determine whether or not, in the absence of endogenous ovarian estrogens, dietary genistein can promote or suppress the growth of neoplastic mammary tissue at various stages for the carcinogenic process.

FY 2000 Accomplishments:

Data collection, except for pathology, was completed.

FY 2001 Plans:

1) Complete compilation of data from pilot study.
2) Initiate tumor study.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Delclos, K. Barry

- ***Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats (Without Behavioral Breeding)*** — ***E0212201*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine the doses of genistein to be used in a multigeneration bioassay for establishing the effects of this naturally occurring isoflavone on development of reproductive organs, reproduction, cancer of the reproductive organs, and neurological and immunological function.

FY 2000 Accomplishments:

1) Manuscripts have been prepared and are currently undergoing internal review.
2) Pathology data completed.

FY 2001 Plans:

The final report and associated manuscripts will be completed.

- ***Range Finding Study for the Evaluation of the Toxicity of Methoxychlor Administered Feed to CD (Sprague-Dawley) Rats*** — ***E0212301*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine the doses of methoxychlor for use in a multigeneration bioassay for assessing the effects of this pesticide on the development of the reproductive tract, reproduction, cancer of the reproductive organs, and neurological and immunological function.

FY 2000 Accomplishments:

In-life completed, but pathology is currently on hold.

FY 2001 Plans:

Depending on resources available, pathology may be completed.

- ***Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats*** — ***E0212501*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine the doses of nonylphenol for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

FY 2000 Accomplishments:

1) Manuscripts have been prepared and are currently undergoing internal review.

2) Pathology data completed.

FY 2001 Plans:

The final report and associated manuscripts will be completed.

- ***Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats*** — ***E0212601*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine the doses of vinclozolin for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

FY 2000 Accomplishments:

In-life completed, currently in pathology.

FY 2001 Plans:

1) Complete pathology.
2) Prepare draft final report and associated manuscripts.

- ***Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats During Development*** — ***E0212901*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine the doses of ethinyl estradiol (EE2) for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

FY 2000 Accomplishments:

1) Manuscripts are being prepared and should undergo internal review by end of the calendar year.
2) Pathology data completed.

FY 2001 Plans:

The final report and associated manuscript will be completed.

- ***Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages*** — ***E0213201*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine the effects of genistein, a naturally occuring isoflavone, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations.

2) To determine if subtle effects observed in the dose-range-finding study are magnified through multiple generations.
3) To evaluate the reversibility of any observed effects.
4) To evaluate the chronic toxicity of genistein, particularly potential induction of cancer of the reproductive organs, following exposures that will include various life stages (*in utero* through early adulthood, *in utero* and continuous for two years after birth, *in utero* and lactational only, and postweaning only).

FY 2000 Accomplishments:

1) Reproductive phase was completed and pathology will be complete the first quarter of 2001.
2) Chronic phase is progressing and will end in the spring of 2001.
3) Analyzed steroid receptor expression in male and female reproductive tracts.
4) Manuscript accepted on blood and tissue levels of genistein.
5) Manuscript being prepared on transplacental transfer of genistein.

FY 2001 Plans:

1) Complete In-life phase of chronic study.
2) Complete pathology and statistical analyses of reproductive phase.
3) Prepare manuscript of steroid receptor data.

◆ ***para-Nonylphenol: Evaluation of Reproductive Effects over Multiple Generations*** — ***E0213501*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine the effects of p-nonylphenol, an intermediate in the production of surfactants and other industrial products, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations.
2) To determine if subtle effects observed in the dose-range-finding study are magnified through multiple generations.
3) To evaluate the reversibility of any observed effects.

FY 2000 Accomplishments:

1) The multigeneration study continued with a scheduled In-life completion in early 2001.
2) Analyzed serum testosterone levels, testicular steroidogenesis, steroid hormone receptor levels.
3) Began preparation of a manuscript of serum testosterone and testicular steroidogenesis.

FY 2001 Plans:

1) The In-life phase will be completed.
2) Initiate pathology and statistical analyses.
3) Complete collection and analysis of steroid hormone receptor expression data.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Ethinyl Estradiol: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages***	***E0213801 E0213821***	***None***	***Agent-Driven***

Objective(s):

1) To evaluate the effects of ethinyl estradiol, a potent synthetic estrogen widely used in prescription drugs, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats in the diet over multiple generations.
2) To determine if subtle effects observed in the dose-range-finding study are magnified through multiple generations.
3) To evaluate the reversibility of any observed effects.
4) To evaluate the chronic toxicity of ethinyl estradiol, particularly the potential induction of cancer of the reproductive organs, following exposures that will include various life stages.

FY 2000 Accomplishments:

1) Protocol was written.
2) Animals will begin being treated at the end of the calendar year.

FY 2001 Plans:

Continue reproductive and chronic studies.

PI: Doerge, Daniel

◆ ***Toxic Hazards from Anti-thyroid Chemicals***	***E0692001***	***None***	***Concept-Driven***

Objective(s):

1) To determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase.
2) To determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray-mass spectrometry to analyze intact adducted proteins and/or proteolytic fragments.
3) To determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/coupling reactions involved in thyroid hormone synthesis.
4) To determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis in rats.

FY 2000 Accomplishments:

Completed manuscript describing the effects of dietary genistein on the rat thyroid.

FY 2001 Plans:

Not Applicable.

Title	Project Number	Collaborator	Strategic Research Goal

- ***Development of Methods for Analysis and Confirmation of b-Agonists*** — ***E0694501*** — ***None*** — ***Method-Driven***

Objective(s):

1) To develop determinative and confirmatory procedures using Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI/MS) for multiresidue screening β-agonists in livestock tissues.
2) To develop synthetic procedures to produce authentic β-agonists standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories.
3) To explore the use of packed column supercritical fluid chromatography (SFC) coupled with APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of β-agonists in livestock tissues.

FY 2000 Accomplishments:

Residue study for ractopamine undertaken in collaboration with the U.S. Department of Agriculture (USDA).

FY 2001 Plans:

Complete ractopamine residue analysis from USDA feeding trial.

- ***Measurement of Oxidative DNA Damage in Normal and Hepatitis C-Infected Human Liver*** — ***E0706401*** — ***None*** — ***Method-Driven***

Objective(s):

1) To develop simple synthetic methods to produce stable labeled analogs of 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG.
2) To develop an automated on-line sample preparation method to maximize detection sensitivity for 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG, in a single sample analysis, using liquid chromatography and tandem mass spectrometry.
3) To apply methodology to the analysis of hepatic DNA from humans and animals.
4) To determine feasibility for application to clinical trials of therapeutic agents and toxicity/carcinogenicity testing in experimental animals.

FY 2000 Accomplishments:

1) Developed sensitive and selective high-throughput LC/MS methods for etheno DNA adducts, 8-oxo-dG, and M1-dG.
2) Two manuscripts were published.

FY 2001 Plans:

1) Complete LC/MS methods development.
2) Apply methodology to analyze oxidative damage in liver DNA using human hepatic, monkey, and rat tissues.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***LC/MS Analysis of Exogenous DNA Adducts***	***X00038***	***None***	***Method-Driven***

Objective(s):

Quantify DNA adducts.

FY 2000 Accomplishments:

Not Applicable.

FY 2001 Plans:

1) Prepare protocol.
2) Continue methods development to quantify DNA adducts from benzo[a]pyrene, tamoxifen, 4-aminobiphenyl, methylanilines, heterocyclic aromatic amines, nucleoside analogues.

◆ ***Effect of Soy Isoflavones on Sensitive Human Populations (Infants and geriatrics)***	***X90023***	***None***	***Agent-Driven***

Objective(s):

To study the effects of soy infant formula on neonatal hormones and sexual development.

FY 2000 Accomplishments:

Not Applicable.

FY 2001 Plans:

1) Write protocol for epidemiological investigation of the effects of soy infant formula on neonatal hormones and sexual development.
2) Use previously developed automated LC/MS methods for analysis of soy isoflavones in human fluids as part of a cancer isoflavone chemoprevention study and to determine possible beneficial or detrimental effects in geriatric patients.

PI: Fu, Peter

◆ ***The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells***	***E0687901***	***CDER***	***Predictive Toxicology***

Objective(s):

1) To determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs.
2) To examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, and aflatoxin B_1.
3) To study the metabolism and DNA adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which, if any, cytochrome P450 is responsible for metabolic activation in mice and humans.

4) Employ transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes to study the mutations and DNA binding of the subject drugs.

FY 2000 Accomplishments:

1) Completed range-finding studies on 22 compounds.
2) Initiated bioassay of benzidine, 2-naphthylamine, malachite green, leucomalachite green, MOCA, 4-nitrobiphenyl, styrene oxide, benzene, glycidol, mitomycin C, ochratoxin A, carboplatin, riddelliine, and dehydroretronecine.
3) N-nitrosonornicotine, fumonisin B_1, aristolochic acid, procarbazine, and 4 proton pump inhibitors (pantoprazole, lansoprazole, omeprazole, and rabeprazole) will be loaded at the beginning of October.
4) Conducted *in vivo* mutagenesis studies using $B_6C_3F_1$ and $Tk^{+/-}$ mice with anti-HIV nucleoside analogues.
5) Made presentation at American Association for Cancer Research (AACR), Meeting of Tumorigenicity and Mutagenicity of Nucleoside Analogues.

FY 2001 Plans:

1) Continue bioassay of benzidine, 2-naphthylamine, malachite green, leucomalachite green, MOCA, 4-nitrobiphenyl, styrene oxide, benzene, glycidol, mitomycin C, ochratoxin A, carboplatin, riddelliine, and dehydroretronecine.
2) Continue bioassay on N-nitrosonornicotine, fumonisin B_1 aristolochic acid, procarbazine, and 4 proton pump inhibitors (pantoprazole, lansoprazole, omeprazole, and rabeprazole).
3) Continue *in vivo* mutagenesis studies with anti-HIV nucleoside analogues using $Tk^{+/-}$ mice.

PI: Howard, Paul

◆ ***The Role of Fumonisin B_1 in Fusarium sp. Tumorigenicity in Rats*** ***E0211101*** ***CVM*** ***Agent-Driven***

Objective(s):

1) To determine the effect of fumonisin B_1 on signal transduction pathways in cultured human esophageal epithelial tissues.
2) To determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of *Fusarium graminearum, Fusarium subglutinans, Fusarium moniliforme* or a combination of the three fungi, using ^{32}P-postlabeling technology.
3) To determine the pharmacokinetics of fumonisin B_1 in $B_6C_3F_1$ mice and F344 rats under conditions similiar to those used in the chronic bioassay and in non-human primates.

FY 2000 Accomplishments:

Project completed.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Submit final publications.

◆ ***Comparative Toxicity of Fumonisin Derivatives in Female $B_6C_3F_1$ Mice*** — ***E0212401*** — ***None*** — ***Agent-Driven***

Objective(s):

To compare the toxicity of several fumonisin derivatives in female $B_6C_3F_1$ mice.

FY 2000 Accomplishments:

This project is complete.

FY 2001 Plans:

Submit final report and publication.

◆ ***The Effects of Chemoexfoliation using α- and β-hydroxy Acids on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Exposed to Simulated Solar Light*** — ***E0213101*** — ***CDRH CFSAN*** — ***Agent-Driven***

Objective(s):

The NIEHS/FDA/NCTR Phototoxicity Research and Testing Laboratory is designed to address the effects of compounds on the induction of skin cancer in mice using light sources that are relevant to humans. Input into the design of the facility has been obtained from experts in phototoxicity and photocarcinogenicity. These experts will continue to provide critical advice on the design of the experimental protocols. As a result, a facility will be developed that will meet the rigors of scientific scrutiny, and will generate data for human health risks from the effects of compounds on light-induced skin cancer. The facility is also designed for expansion to allow simultaneous examination of the toxicity or cocarcinogenicity of compounds in the presence of either simulated sunlight or fluorescent UVB light. The mechanistic studies in this proposal will provide the data necessary to design and interpret properly the future alpha-hydroxy acid and simulated solar light cocarcinogenicity.

FY 2000 Accomplishments:

1) Construction of the phototoxicity facility was completed.
2) All animals were loaded to study the effects of α-hydroxy and β-hydroxy acids on SKH-1 mouse skin and to determine the dose-response for the induction of skin edema on SKH-1 mouse skin.
3) Assays developed to measure DNA photoproducts and 8-oxoguanine.

FY 2001 Plans:

1) Continue analysis of DNA photoproducts and 8-oxoguanine.
2) Monitor solar light-induced decomposition of glycolic and salicylic acids.
3) Repeat two short-term studies.

Title	Project Number	Collaborator	Strategic Research Goal

◆ ***Effect of Topically Applied Skin Creams Containing Glycolic and Salicyclic Acid on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice*** — ***E0213701*** — ***CDRH CFSAN*** — ***Agent-Driven***

Objective(s):

To determine if the application of creams containing α- and β-hydroxy acids to the skin of male and female SKH-1 hairless mice alters the tumor incidence induced by simulated solar light in the mouse skin.

FY 2000 Accomplishments:

Animals were loaded in July and will continue to August 2001.

FY 2001 Plans:

Continue bioassay.

◆ ***Comparative Toxicity of Fumonisin B_1 in Rat Strains*** — ***X00040*** — ***None*** — ***Agent-Driven***

Objective(s):

The administration of fumonisin B_1 to F344/N/NCTR Br rats resulted in renal tumors at two years, and no liver lesions. Administration of fumonisin B_1 in South Africa to BD IX resulted in liver tumors in two years, and formation of hepatocellular foci in F344 rats in six months. The goals of this project are:

1) To prepare diets containing fumonisin B_1.
2) To feed male F344/N/NCTR BR, F344, and SD rats the diet for up to six months.
3) To send the same diets to South Africa where BD IX and F344 rats will be fed the diets.
4) The formation of hepatocellular preneoplastic altered enzyme foci, hepatocellular morphology, and hepatocellular enzyme changes will be compared to determine why the different strains gave different carcinogenesis endpoints.

FY 2000 Accomplishments:

Not Applicable.

FY 2001 Plans:

Prepare protocol and initiate studies.

◆ ***Identification of Fusarium Mycotoxin Responsible for DNA Adducts in Human Esophageal Tissue*** — ***X10024*** — ***None*** — ***Concept-Driven***

Objective(s):

A unique DNA adduct has been detected in esophageal tumor tissue taken from South African patients suffering from terminal esophageal cancer. This same adduct can be formed *in vitro* by incubating methanolic extracts of *Fusarium* fungi with DNA. The

fact that the presence of the fungi in food is epidemiologically associated with esophageal cancer suggests the adduct may be involved in cancer formation, or at the least, as a biomarker of exposure. The goals of this project are:

1) To isolate and identify the DNA adduct.
2) To identify the mycotoxin responsible for adduct formation.
3) To determine the extent of the presence of this mycotoxin in foods and the DNA adduct in esophageal tissue samples.

FY 2001 Plans:

1) Prepare protocol.
2) Initiate the studies on DNA adduct identification.

PI: James-Gaylor, Sandra

- ***Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy*** — ***E0700301*** — ***None*** — ***Concept-Driven***

Objective(s):

1) In nitroso methylurea (NMU)-initiated mammary epithelial cells, determine whether nutritional manipulation of the cell cycle combined with low dose chemotherapy will permanently eliminate p53-independent and p53-dependent preneoplastic and neoplastic cells.
2) Determine the mechanisms of cell death induced by nutritional manipulation and low dose chemotherapy by examining molecular endpoints associated with p53-dependent and independent pathways of apoptosis.

FY 2000 Accomplishments:

1) Completed two experiments on nutritional intervention after NMU exposure.
2) Histological samples are currently being processed.

FY 2001 Plans:

1) Complete histological processing of samples.
2) Prepare final report and manuscripts.

- ***Molecular and Metabolic Determinants of Maternal Risk and Progression of Down Syndrome: Potential for Nutritional Interventions*** — ***E0701601*** — ***None*** — ***Concept-Driven***

Objective(s):

1) To define abnormalities in one-carbon metabolism in mitogen-stimulated lymphocytes from women who have had a child with Down Syndrome and to determine whether appropriate folate/methyl supplementation can normalize these metabolic abnormalities.

2) To define the biochemical and molecular consequences of abnormal one-carbon metabolism in mitogen-stimulated lymphocytes from Down Syndrome children and to determine whether these metabolic abnormalities can be normalized with targeted nutritional intervention.

FY 2000 Accomplishments:

Four papers focusing on abnormal folate metabolism, mutations in the MTHFR gene, and Down Syndrome were accepted for publication.

FY 2001 Plans:

1) Continue collaborations involving measurements of thiols and DNA methylation in mothers of children with Down Syndrome to determine whether an increased homocysteine is a maternal risk factor.
2) Use expression array technology to determine tissue-specific gene expression in various tissues obtained at autopsy from infants with Down Syndrome.
3) Continue development of HPLC/EC methodology to evaluate folate metabolites in plasma and tissue.

◆ ***DNA Damage with Dietary Methyl Donor Deficiency*** — ***E0706501*** — ***None*** — ***Concept-Driven***

Objective(s):

To further the understanding of the mechanisms by which diet, as an environmental variable, can alter the susceptibility to cancer.

FY 2000 Accomplishments:

Four papers focusing of uracil misincorporation, DNA strand breaks, gene amplification, and abnormal methylation patterns were accepted for publication.

FY 2001 Plans:

1) Use cDNA array technology to establish nutritionally induced alterations in gene expression during various stages of neoplasia.
2) Examine relationship between nuclear levels of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) and DNA methylation.
3) Determine whether or not site-specific DNA stand breaks and/or mutations in promoter regions of p53 and p16 induce reduced expression of these genes.

PI: Roberts, Dean

◆ ***Antigenic Biomarkers of Estrogen Catechol Metabolism for Use in Epidemiological Studies*** — ***E0705701*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To prepare immunogenic conjugates for immunization of rabbits and antigenic conjugates for the characterization of antisera and for affinity purification of antibodies.

2) To develop IA/LC/MS methods to detect the antigenic biomarkers in urine and/or serum.
3) To initiate studies to validate the use of the antibody reagents and IA/LC/MS methods developed in Objectives 1 and 2 using human urine and serum samples collected in an ongoing study of reproductive events, carcinogen metabolism, and interindividual variability.

FY 2000 Accomplishments:

1) Developed HPLC EC detection for estrogen metabolites (E3, 16-OH-E1, 4-OH-E2, 2-OH-E1, E2, 4-Me-E2, 2-Me-E2, E1, 4-Me-E1, and 2-Me-E1).
2) Validated on-line solid-phase extraction for urine samples.
3) Established collaborations to assay tissue culture samples.
4) Made presentations at American Association for Cancer Research (AACR) and Society of Toxicology (SOT) meetings.

FY 2001 Plans:

Continue experiments as outlined in protocol.

◆ ***Development of On-line Proteomics Employing Automated On-line Columnswitching for Two-dimensional Mapping, Selective and Subtractive Mapping and Isolation of Proteins as a Function of Genotype, Phenotype, and Toxicant Exposure*** — ***X10025*** — ***None*** — ***Method-Driven***

Objective(s):

None submitted.

FY 2000 Accomplishments:

Not applicable.

FY 2001 Plans:

1) Prepare protocol.
2) Initiate experiments.

PI: Tolleson, William

◆ ***Purification of Ceramide Synthase*** — ***E0705901*** — ***None*** — ***Concept-Driven***

Objective(s):

1) To isolate rat ceramide synthase.
2) To identify the gene coding for rat ceramide synthase.
3) To develop antibodies to rat ceramide synthase.
4) To use the antibodies to study tissue specific expression of ceramide synthase.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) Several chromatographic techniques including cation exchange, anion exchange, hydroxy apatite, and hydrophobic chromatography were evaluated.
2) A new technique based upon affinity chromatography has been developed.
3) One paper has been submitted for publication.

FY 2001 Plans:

1) Continue purification based upon affinity chromatography.
2) Determine partial amino acid sequence, prepare degenerate oligomeric sequences, and probe cDNA libraries.
3) Prepare expression vector for producing recombinant ceramide synthetase.
4) Develop ceramide synthase-specific antibodies to study expression of the enzyme in various tissues.

FY 2000 Publications

Bajic, S., Doerge, D.R., Lu, L. and Hansen, E., LC/MS Analysis of Erythromycin Using Involatile Mobile Phases with a Novel API Ion Source, Rapid Communications in Mass Spectrometry, 14:156-160. Accepted: 11/30/1999 **(NA)**.

Banfalvi, G., Littlefield, N.A., Hass, B.S., Mikhailova, M.V., Csuka, L. and Chou, M.W., Effect of Cadmium on the Relationship Between Replicative and Repair DNA Synthesis in Synchronized CHO Cells, European Journal of Biochemistry, 267:6580-6585. Accepted: 9/8/2000 **(E0696701)**.

Bever, R.J., Couch, L.H., Sutherland, J.B., Williams, A.J., Beger, R., Churchwell, M.I., Doerge, D.R. and Howard, P., DNA Adduct Formation by *Fusarium* Culture Extracts: Lack of Role for Fusarin C., Chemico-Biological Interactions, 128:141-157. Accepted: 8/2/2000 **(E0211101)**.

Chang, C., Churchwell, M.I., Delclos, K.B. and Doerge, D.R., Mass Spectrometric Determination of Genistein Tissue Distribution in Sprague Dawley Rats from Dietary Exposure, Journal of Nutrition, 130:1963-1970. Accepted: 3/1/2000 **(E0213201)**.

Chang, C., Doerge, D.R., Dietary Genistein Causes Inactivation of Rat Thyroid Peroxidase *In Vivo* Without an Apparent Hypothyroid Effect, Toxicology and Applied Pharmacology, 168:244-252. Accepted: 7/21/2000 **(E0213531)**.

Chen, R., Chou, M.W. and Ueng, T., Induction of Cytochrome P450 1A1 in Human Hepatoma HepG2 Cells by Nitrochrysene, Toxicology Letters, 117:69-77. Accepted: 7/18/2000 **(E0260001)**.

Chen, Z., Pogribny, I.P., Melnyk, S.B., Bottiglieri, T., Selhub, J.J. and James-Gaylor, S.J., Mice Deficient in Methylenetetrahydrofolate Exhibit Hyperhomocysteinemia and Decreased Methylation Capacity, Human Molecular Genetics. Accepted: 10/1/1999 **(NA)**.

Cho, B.P., Blankenship, L., Moody, J.D., Doerge, D.R., Beland, F.A. and Culp, S.J., Synthesis and Characterization of 4'-amino and 4'-nitro Derivatives of 4-N,N-dimethylaminotriphenylmethane as Precursors for a Proximate Malachite Green Metabolite, Tetrahedron, 56:7379-7388. Accepted: 7/22/2000 **(E0212801)**.

Chou, M.W., Mikhailova, M.V., Nichols, J.A., Poirier, L.A., Warbritton, A.R. and Beland, F.A., Interactive Effects of Methyl-deficiency and Dietary Restriction on Liver Cell Proliferation and Telomerase Activity in Fischer 344 Rats Treated with Aflatoxin B_1, Cancer Letters, 152 (1):53-61. Accepted: 12/1/1999 **(E0695201)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Chung, K., Wong, T., Li, Y.S., Wei, C.I. and Chou, M.W., Mutagenicity Studies of Benzidine and its Analogs: Structure-Activity Relationships, Toxicological Sciences, 56:351-356. Accepted: 3/29/2000 **(E0260001)**.

Coles, B.F., Anderson, K., Doerge, D.R., Churchwell, M.I., Lang, N.P. and Kadlubar, F.F., Quantitative Analysis of Inter-individual Variation of Glutathione S-Transferase Expression in Human Pancreas and the Ambiguity of Correlating Genotype with Phenotype, Cancer Research, 60(3):573-579. Accepted: 12/1/1999 **(E0699001)**.

Culp, S.J., Warbritton, A.R., Smith, B.A., Li, E. and Beland, F.A., DNA Adduct Measurements, Cell Proliferation, and Tumor Mutation Induction in Relation to Tumor Formation in $B_6C_3F_1$ Mice Fed Coal Tar or Benzo[a]pyrene, Carcinogenesis, 21:1433-1440. Accepted: 3/29/2000 **(E0672202)**.

Dalu, A.N., Blaydes, B.J., Lomax, L.G. and Delclos, K.B., A Comparison of the Inflammatory Response to a Polydimethylsiloxane Implant in Male and Female Balb/c Mice, Biomaterials, 21:1947-1957. Accepted: 3/13/2000 **(E0696301)**.

Doerge, D.R., Chang, C., Churchwell, M.I. and Holder, C.L., Analysis of Soy Isoflavone Conjugation *In Vitro* and in Human Blood Using LC/MS, Drug Metabolism and Disposition, 28(3):298-307. Accepted: 11/9/1999 **(NA)**.

Doerge, D.R., Churchwell, M.I. and Delclos, K.B., On-Line Sample Preparation Using Restricted-Access Media in the LC-ES/MS Analysis of the Soy Isoflavones, Genistein and Daidzein, in Rat Serum, Rapid Communications in Mass Spectrometry, 14:673-678. Accepted: 2/28/2000 **(E0213201)**.

Doerge, D.R., Churchwell, M.I., Fang, J. and Beland, F.A., Quantification of Etheno-DNA Adducts Using On-line Sample Preparation and LC-ES/MS/MS, Chemical Research in Toxicology, 13:1259-1264. Accepted: 9/1/2000 **(E0212001)**.

Doerge, D.R., Fogle, C.M., Paule, M.G., McCullagh, M. and Bajic, S., Analysis of Methylphenidate and its Metabolite Ritalinic Acid in Monkey Plasma by Liquid Chromatography/Electrospray Ionization Mass Spectrometry, Rapid Communications in Mass Spectrometry, 14:619-623. Accepted: 2/4/2000 **(E0692001)**.

Fang, G., Chang, C., Wu, Y., Fu, P.P. and Chen, S., The Study of TSP, PM2.5-10 and PM2.5 During Taiwan Chi-Chi Earthquake in the Traffic Site of Central Taiwan, Taichung, Chemosphere, 41:1727-1731. Accepted: 2/10/2000 **(E0657300)**.

Fang, G., Chang, C., Wu, Y., Fu, P.P., Yang, D. and Chen, S., Comparison of Particulate Mass, Chemical Species for Urban, Suburban and Rural Areas in Central Taiwan, Taichung, Chemosphere, 41:1349-1359. Accepted: 12/16/1999 **(E0657300)**.

Fang, G., Chang, C., Wu, Y., Fu, P.P., Yang, D. and Chen, S., Study on Particulate and Metallic Elements Variation at Daytime and Nighttime Period in Urban Atmosphere, Toxicol. Environ. Chem, 76:83-94. Accepted: 1/1/2000 **(E0657300)**.

Fang, G., Chang, C., Wu, Y., Fu, P.P., Yang, D., Chen, S. and Chu, C., The Study of Fine and Coarse Particles, and Metallic Elements for the Daytime and Nighttime in a Suburban Area of Central Taiwan, Taichung, Chemosphere, 41:639-644. Accepted: 10/11/1999 **(E0657300)**.

Fang, J., Beland, F.A., Development of a Novel ^{32}P-postlabeling Method for the Analysis of 3'-azido-3'-deoxythymidine, Cancer Letters, 153:25-33. Accepted: 12/20/1999 **(E0687901)**.

Ferguson, S.A., Flynn, K.M., Delclos, K.B. and Newbold, R., Maternal and Offspring Toxicity but Few Sexually Dimorphic Behavioral Alterations Results from Nonylphenol Exposure, Neurotoxicology and Teratology, 22:583-591. Accepted: 1/14/2000 **(E0212513)**.

Flynn, K.M., Ferguson, S.A., Delclos, K.B. and Newbold, R., Effects of Genistein Exposure on Sexually Dimorphic Behaviors, Toxicological Sciences, 55 (2):311-319. Accepted: 2/4/2000 **(E0212213)**.

Flynn, K.M., Ferguson, S.A., Delclos, K.B. and Newbold, R., Multigenerational Exposure to Genistein has Minimal Effects on Maternal Behavior in Rats, NeuroToxicology. Accepted: 7/5/2000 **(E0213213)**.

Fu, P.P., Von Tungeln, L.S., Hammons, G.J., McMahon, G., Wogan, G., Flammang, T.J. and Kadlubar, F.F., Metabolic Activation Capacity of Neonatal Mice in Relation to the Neonatal Mouse Tumorigenicity Bioassay, Drug Metabolism Reviews, 32 (2):241-266. Accepted: 12/9/1999 **(E0687901)**.

Gamboa da Costa, G., Hamilton, L.P., Beland, F.A. and Marques, M.M., Characterization of the Major DNA Adduct Formed by Alpha-hydroxy-N-desmethyltamoxifen *In Vitro* and *In Vivo*, Chemical Research Toxicology, 13:200-207. Accepted: 1/28/2000 **(E0701101)**.

Herreno-Saenz, D., Von Tungeln, L.S., Hart, R.W. and Fu, P.P., Effect of Dietary Restriction and Age on the Formation of DNA Adducts from the Mouse Liver Microsome-Mediated Metabolism of 2-Nitropyrene, Polycyclic Aromatic Compounds, 16:151-159. Accepted: 10/5/1999 **(E0657300)**.

Hong, F., Von Tungeln, L.S. and Fu, P.P., Stereoselective Metabolism of Anthracene, 9-methylanthracene, 9-10-dimethylanthracene, 9-chloroanthracene, and 9-nitroanthracene by Liver Microsomes of 15-day-old Male $B_6C_3F_1$ Mice, Polycyclic Aromatic Compounds, 16:235-244. Accepted: 10/18/1999 **(E0657300)**.

Howard, P., Eppley, R., Stack, M.E., Warbritton, A.R., Voss, K.A., Lorentzen, R., Kovatch, R. and Bucci, T.J., Fumonisin B_1 Carcinogenicity in a Two-year Feeding Study Using F344 Rats and $B_6C_3F_1$ Mice, Environmental Health Perspectives. Accepted: 8/16/2000 **(E0210601, E0210801)**.

Howard, P., Eppley, R., Warbritton, A.R., Voss, K.A., Lorentzen, R., Kovatch, R.M. and Bucci, T.J., Carcinogenicity of Fumonisin B_1 in a Two-year Bioassay with Fischer 344 Rats and $B_6C_3F_1$ Mice, Mycotoxins (Japan) Meeting Proceedings, S:45-54. Accepted: 10/15/1999 **(E0210601)**.

Howard, P., Warbritton, A.R., Voss, K.A., Lorentzen, R., Thurman, J.D., Kovatch, R. and Bucci, T.J., Compensatory Regeneration as a Mechanism for Renal Tubule Carcinogenesis of Fumonisin B_1 in the F344/N/NCTR BR Rat, Environmental Health Perspectives. Accepted: 8/16/2000 **(E0210601, E0210801)**.

James-Gaylor, S.J., Hobbs, C.A. and Hine, R., Folate Deficiency and the Molecular Determinants of Chromosomal Instability: Possible Link to Down Syndrome and Meiotic Nondisjunction, Folate and Human Development, In Press. Accepted: 8/1/2000 **(NA)**.

Jenkins, R.E., Tolleson, W.H., Newkirk, D.K., Roberts, D.W., Rowland, K.L., Howard, P. and Melchior, W.B., Identification of Fumonisin B_1 as an Inhibitor of Argininosuccinate Synthetase using Fumonisin Affinity Chromatography and *In Vitro* Kinetics Studies, Journal of Biochemistry and Molecular Toxicology, 14:320-328. Accepted: 6/5/2000 **(E0211001, E0705901)**.

Kodell, R.L., Young, J.F., Delongchamp, R.R., Turturro, A., Chen, J.J., Gaylor, D.W., Howard, P. and Zheng, Q., A Mechanistic Approach to Modeling the Risk of Liver Tumors in Mice Exposed to Fumonisin B_1 in the Diet, Food Additives and Contaminants. Accepted: 5/2/2000 **(S00174)**.

Li, Y.S., Fu, P.P. and Church, J., The Conformation of Some Nitro-polycyclic Aromatic Hydrocarbons, J. Molecular Structure, 550:217-223. Accepted: 11/17/1999 **(E0657300)**.

Manjanatha, M., Shelton, S.D., Culp, S.J., Blankenship, L. and Casciano, D.A., DNA Adduct Formation and Molecular Analysis of *In Vivo LacI* Mutations in the Mammary Tissue of Big Blue Rats Treated with 7,12-dimethylbenz[a]anthracene, Carcinogenesis, 21:265-273. Accepted: 10/8/1999 **(E0690611)**.

Melnyk, S.B., Pogribna, M.V., Pogribny, I.P., Yi, P. and James-Gaylor, S.J., Measurement of Plasma and Intracellular S-adenosylmethionine and S-adenosylhomocystein Utilizing Coulometric Electrochemical Detection: Alterations with Plasma Homocystein and Pyridoxal 5'-phosphate levels, Clinical Chemistry, 46(2):265-272. Accepted: 12/1/1999 **(E0701601)**.

Mittelstaedt, R.A., Smith, B.A., Beland, F.A. and Heflich, R.H., Sequence Specificity of *Hprt* Lymphocyte Mutation in Rats Fed the Hepatocarcinogen 2-acetylaminofluorene, Mutation Research, 431:167-173. Accepted: 10/5/1999 **(E0697021)**.

Phillips, D.H., Farmer, P.B., Beland, F.A., Nath, R.G., Poirier, M.C., Reddy, M.V. and Turteltaub, K.W., Methods of DNA Adduct Determination and Their Application to Testing Compounds for Genotoxicity, Mutation Research, 35:222-233. Accepted: 10/5/1999 **(S00189)**.

Pogribny, I.P., Pogribna, M.V., Christman, J.K. and James-Gaylor, S.J., Single Site Methylation Within the p53 Promoter Region Reduces Gene Expression in a Reporter Gene Construct: Possible *In Vivo* Relevance During Tumorigenesis, Cancer Research, 60 (3):588-594. Accepted: 11/1/1999 **(E0687501)**.

Sahu, S., Chou, M.W., Sotomayer, R., Hinton, D. and Barton, C.N., Effects of Intermittent Exposures of Aflatoxin B_1 on Hepatic and Testicular Glutathione S-Transferase in Rats, Journal of Applied Toxicology, 20 (3):215-219. Accepted: 12/1/1999 **(E0688801)**.

Sams, R.L., Blaydes, B.J., Warbritton, A.R., Lomax, L.G., Bucci, T.J., Muskhelishvili, L. and Delclos, K.B., Differences in the Response to Oxidative Stress and Mutation Frequency in CD (Sprague-Dawley) and Fisher 344 Rats Due to an Induced Inflammatory Response, Environmental and Molecular Mutagenesis, 35 (4):336-342. Accepted: 10/23/1999 **(E0699301)**.

Yang, Y., Yan, J., Churchwell, M.I., Beger, R., Chan, P.C., Doerge, D.R., Fu, P.P. and Chou, M.W., Development of a ^{32}P-Postlabeling/HPLC Method for Detection of Dehydroretronecine-Derived DNA Adducts *In Vivo* and *In Vitro*, Chemical Research in Toxicology, 14:91-100. Accepted: 11/1/1999 **(E0213301)**.

Yang, Y., Yan, J., Doerge, D.R., Chan, P.C., Fu, P.P. and Chou, M.W., Metabolic Activation of the Tumorigenic Pyrrolizidine Alkaloid Riddelliine, Leading to DNA Adduct Formation *In Vivo*, Chemical Research in Toxicology, 14:101-109. Accepted: 11/1/1999 **(E0213301)**.

Yi, P., Melnyk, S.B., Pogribna, M.V., Pogribny, I., Hine, R. and James-Gaylor, S.J., Increase in Plasma Homocysteine Associated with Parallel Increases in Plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation, Journal of Biological Chemistry, 275:29318-323. Accepted: 3/1/2000 **(E0701601)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

BIOMETRY AND RISK ASSESSMENT

Director: ***Ralph L. Kodell, Ph.D.***
Telephone: **870-543-7008**
Toll Free: **800-638-3321**
E-mail: **rkodell@nctr.fda.gov**

Dr. John Young modeling differential equations on a hybrid computer.

Executive Summary

During FY 2000, scientists in the Division of Biometry and Risk Assessment conducted research in a number of areas relevant to science-based regulations, and initiated research in several important new areas. Protocols were either initiated or implemented to conduct research on: assessing the risk of infection and disease from food-borne pathogens; predicting the additive risk of health effects from mixtures of toxic chemicals; developing computer-based systems to predict toxicity of untested agents; assessing skin cancer risk due to interactions of cosmetics with sunlight; and interpreting gene expression data from DNA microarrays. These research efforts are directed toward addressing both presently defined FDA safety initiatives and future FDA needs in informatics and biotechnology.

A major effort completed by the Division in FY 2000 was the development and application of a biologically based model to predict cancer risk in mice after exposure to fumonisin B_1 in the diet. Fumonisin B_1, which arises from a fungus that grows on corn, is a contaminant of corn-based food products and must be regulated to low levels. A complex mathematical cancer model was formulated and implemented by the Division's Dr. Qi Zheng, based on data from NCTR's study of fumonisin B_1. All Division scientists and staff participated in the risk assessment. The model predicted negligible liver tumor risk at low exposure levels in both male and female mice, predicting substantially increased risk above background risk only at the highest doses of fumonisin B_1 in females. Results were presented at FDA's Workshop on Risk Assessment of Fumonisins.

A collaborative effort with scientists in EPA's National Center for Environmental Assessment was established in FY 2000 to study infection by the protozoan *Cryptosporidium parvum*, a common contaminant of drinking water that can also contaminate the food supply. Uncertainty regarding the number of oocysts required to induce infection impacts decisions on acceptable regulatory levels of this protozoan in food and water. A research protocol to study the transmission dynamics of infection by *Cryptosporidium parvum* has been developed by the Division's Dr. Angelo Turturro, with input from scientists at FDA's Center for Food Safety and Applied Nutrition (CFSAN). The protocol should be implemented in FY 2001.

Included in the interagency agreement with EPA is the development of a quantitative strategy to predict the risk of adverse health effects from exposure to chemical mixtures.

In particular, organophosphate insecticides are of concern because of their widespread use in combination, including house and garden use, and because of the potential for their adverse respiratory and central nervous system (CNS) effects to be additive. A research protocol to develop a strategy to quantify the additive risk was begun by the Division's Dr. James Chen in FY 2000 and will be finalized and implemented in FY 2001. The Division was fortunate to recruit a post graduate statistician, Ms. Yi-Ju Chen, to collaborate in this research.

FDA's growing need to bring safe products to market more quickly, while ensuring a reliable process for screening out unsafe products, has highlighted the need for computer-based systems to evaluate the safety of products that might not undergo full toxicity testing. In response to this need, the Division's Dr. John Young initiated a protocol in FY 2000 to develop a computational predictive system for rodent organ-specific carcinogenicity. This work will be done in conjunction with scientists from various research programs at NCTR, as well as in collaboration with CFSAN. The aim of the project is to combine chemical structure data, spectral data, and short-term toxicity data to classify chemicals with respect to their potential to cause cancer in specific target organs, such as the liver. The protocol, which will be implemented in FY 2001, intends to provide FDA with a reliable computational tool that might allow the Agency to streamline toxicity-testing requirements for new products, e.g., by requiring sponsors to conduct only target-specific toxicity testing based on the system's prediction.

The effort to develop new statistical theory and methods for assessing skin cancer risk continued under the direction of Dr. Ralph Kodell, in conjunction with the Center's NTP phototoxicity research program directed by Dr. Paul Howard of the Division of Biochemical Toxicology. Photocarcinogenicity has become increasingly important to FDA. The fact that most animals develop multiple skin tumors in photocarcinogenicity studies presents statistical challenges not encountered with conventional animal bioassays, where single tumors of a given type are most commonly observed. In addition to developing enhanced methods of analysis, this research involves comparisons with conventional methods, using both data from simulated experiments and data obtained through a cooperative agreement with Argus Laboratories. This effort has been enhanced by the recruitment of post-doctoral fellow, Dr. Daniel Molefe.

A new biotechnological tool that has captivated the scientific community is the gene microarray. Vast amounts of data on gene expression and genetic profiles can be obtained quickly using such arrays. However, there are still many uncertainties regarding the proper design and construction of these arrays, as well as the analysis and interpretation of the resulting data. In FY 2000, the Division's Dr. Robert Delongchamp initiated development of a protocol to provide sound statistical theory and methods for assessing differences in gene expression using microarray data. This work is being conducted in collaboration with genetic toxicologist Ms. Angela Harris of the Division of Genetic and Reproductive Toxicology. The effort has been enhanced by the establishment of a part-time NCTR appointment for Dr. Richard Evans, a biostatistician at the University of Arkansas for Medical Sciences (UAMS) who has expertise in analyzing microarray data in a clinical setting, and by the recruitment of a post-doctoral

fellow, Dr. Cruz Velasco. Having sound analytical methods for gene microarrays and other emerging technologies is a critical FDA regulatory need.

The Division's Mr. John Appleget continues to serve as co-project officer for the Center's Information Technology contract, coordinating biostatistical and experimental-liaison activities. Ms. Susan Taylor ensures that all of the Division's administrative systems function smoothly, and Mr. Bruce Pearce provides scientific programming support for a number of research projects. Information on other Division projects not described here may be found under the FY 2000 and 2001 Accomplishments and Plans.

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Chen, James

- ***Analysis of Multiple Tumor Sites*** — ***E0700901*** — ***None*** — ***Method-Driven***

Objective(s):

1) To develop analytical and numerical techniques for computing the experiment-wise error rate in testing of multiple tumor sites.
2) To evaluate and compare the experiment-wise error rate and power of various methods of p-value adjustment and recommend an optimal method for test of site-specific effects.
3) To evaluate the experiment-wise error rate and power of global statistics for an overall test of carcinogenicity.
4) To recommend optimal procedures which control the experiment-wise error rate and still maintain the power for the analysis of multiple tumor sites.

FY 2000 Accomplishments:

1) One paper on "weighted adjustment method for simultaneous analysis of multiple tumor sites/types" was published.
2) One paper on "a sequential closed testing procedure for comparisons of dose groups with control" was published.
3) An invited paper was presented at the 2nd International Conference on Multiple Comparisons.

FY 2001 Plans:

Resubmit the manuscript on "global-based statistics to test for subsets of multiple endpoints," a collaborative publication with CDER.

- ***Dose-Response Modeling for Microbial Risk Assessment*** — ***E0704501*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To evaluate existing dose-response models for microbial risk assessment.
2) To develop improved models for estimating probabilities of infection and disease.
3) To develop methods for incorporating model uncertainty into microbial risk assessment.

FY 2000 Accomplishments:

1) One paper on a new approach to incorporating model uncertainties for risk assessment was accepted for publication.
2) One paper on "statistical models for microbial risk assessment" has been submitted for publication.
3) An invited paper was presented at the Eastern North American Region (ENAR) of the International Biometric Society.
4) An invited talk was presented at the Toxicology and Risk Assessment Approaches for the 21st Century.

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

5) Recruited ORISE post doc.

FY 2001 Plans:

1) Continue research on dose-response modeling of microbial risk assessment.
2) Evaluate the approach to incorporating model uncertainties in risk estimation.

◆ ***Tests of Equivalence for Dichotomous Endpoints*** — ***E0706201*** — ***CDER*** — ***Predictive Toxicology***

Objective(s):

1) To investigate the size of the asymptotic and unconditional exact tests for assessing equivalence between two binomial proportions in 2×2 tables.
2) To propose an approximate exact test for assessing equivalence between two binomial proportions.
3) To compare the approximate exact test with the asymptotic and unconditional exact tests in terms of the size and power.
4) To develop the asymptotic, unconditional, and approximate exact tests of equivalence for the logistic regression trend test in 2×k tables.
5) To develop the asymptotic, unconditional, and approximate exact tests of equivalence for two multinomial proportions in k×2 tables.

FY 2000 Accomplishments:

1) Two papers were published.
2) Two manuscripts were submitted for publication.
3) Presented an invited talk at the FDA session, International Chinese Statistical Association (ICSA) Applied Statistics Symposium.

FY 2001 Plans:

1) Continue collaborative research on the development of procedures for comparing two diagnostic test procedures.
2) Continue collaborative research on the procedures for multinomial outcomes.

PI: Delongchamp, Robert

◆ ***Mortality Among Atomic Bomb Survivors who were Exposed In Utero*** — ***E0702901*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To estimate the dose-response relationship between non-cancer mortality and radiation exposure.
2) To assess the effect of gestational age at exposure on mortality.
3) To appraise the role of severe mental retardation in mortality.

FY 2000 Accomplishments:

1) Manuscript prepared for publication.
2) Attended the American Statistical Association Conference on Radiation and Health, Park City, UT.

FY 2001 Plans:

1) Complete publication of manuscripts on health effects in atomic-bomb survivors.
2) Complete project/final report.

◆ ***A Mixture Model Approach to Classifying CYP1A2 Variants that Adjusts for their Current Smoking Status*** — ***E0703701*** — ***None*** — ***Method-Driven***

Objective(s):

1) To examine statistical methods for parametric density estimation based upon a mixture of normal distributions.
2) To apply the method to a data set where hepatic cytochrome P4501A2 activity appears to be induced by smoking cigarettes.

FY 2000 Accomplishments:

1) Programmed the method and successfully applied it to the CYP1A2 data.
2) Applied the model to phenotype data on NAT2. Genotyping on the subjects confirmed that this model accurately estimated the frequencies of genotypes involving the wild type allele.
3) Applied the model to phenotype data on SULT1A1. The model predicts a genotype whose frequency is altered among cases. However, the examined polymorphisms are not the predicted genotype.

FY 2001 Plans:

1) Submit a manuscript illustrating the method.
2) Complete project/final report.

◆ ***A Statistical Model to Estimate the In Vivo Mutation Frequency in Mice with FX174 Transgene*** — ***X10018*** — ***None*** — ***Method-Driven***

Objective(s):

To develop statistical methodology for estimating the *in vivo* mutation rate in transgenic mice based on the negative binomial distribution for the burst size, i.e., the number of phages released from an infected bacterium.

FY 2000 Accomplishments:

1) Developed a concept paper for a protocol.
2) Prepared two manuscripts on estimating *in vivo* mutation rates.
3) Recruited ORISE post doc.

FY 2001 Plans:

1) Develop a research protocol.
2) Continue research.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Assessing Differences in Gene Expression from Microarray Data***	***X10019***	***None***	***Method-Driven***

Objective(s):

To develop statistical methods for detecting differences in gene expression as measured on two microarrays, including adjusting for nuisance sources of bias and variation.

FY 2000 Accomplishments:

1) Developed a concept paper for a protocol.
2) Prepared a manuscript on the statistical analysis of gene microarray data.

FY 2001 Plans:

1) Develop a research protocol.
2) Continue research.

PI: Kodell, Ralph

◆ ***Attribution of Tumor Lethality in the Absence of Cause-of-Death Information***	***E0689601***	***None***	***Method-Driven***

Objective(s):

1) To develop a nonparametric procedure for estimating distributions of time to onset of, and time to death from, occult tumors in the absence of cause-of-death information.
2) To develop a method for entering the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the International Agency for Cancer Research (IARC) cause-of-death test.
3) To develop a procedure for estimating the lag time between onset of, and death from, an occult tumor when cause-of-death data are unavailable.
4) To illustrate the new procedures using data from the Project on Caloric Restriction (PCR) studies.

FY 2000 Accomplishments:

1) Paper published on imputing cause of death for occult tumors.
2) Work continued on the development of a modified Peto "cause-of-death" test based on imputed cause of death instead of pathologist-assigned cause of death. Manuscript is in revision.
3) Manuscript is in preparation on estimating tumor latency for occult tumors when cause-of-death information is not available or is unreliable.
4) A talk was presented at the Eastern North American Region (ENAR) of the International Biometric Society meeting.

FY 2001 Plans:

1) Continue research on imputed cause of death.
2) Resubmit revised manuscript on modified cause-of-death test and submit new manuscript on estimation of latency.

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

3) Present an invited talk at the Canadian Conference on Applied Statistics in Montreal.

◆ ***Statistical Analysis of Tumor Multiplicity Data*** ***E0706101*** ***CDER*** ***Predictive Toxicology***

Objective(s):

1) To investigate the model of Kokoska, et al., for analyzing tumor multiplicity data from single-induction experiments, using the negative binomial distribution for the number of induced tumors and the Weibull distribution for the time to observation of such tumors.
2) To develop a likelihood-ratio approach, adapted from the model of Kokoska, et. al., for testing between-group differences with respect to the expected number of induced tumors as well as the distribution of time to observation.
3) To develop tests for dose-related trend with respect to the expected number of induced tumors and the distribution of time to observation.
4) To extend the model to situations involving multiple or continuous dosing, and situations in which there is a background of spontaneous tumors.
5) To conduct a Monte Carlo simulation study to compare the new methodoloy to conventional analytical approaches, and to evaluate its robustness and identifiability.
6) To develop user-friendly software for easy implementation of the proposed analytical procedures.

FY 2000 Accomplishments:

1) Manuscript revised and resubmitted on testing for increased tumor frequency and/or decreased latency in a treated group relative to a control group.
2) Developed statistical testing procedure for multiple-dosing (e.g., phototoxicity) experiments.
3) Presented a departmental seminar on this topic at State University of New York at Stony Brook (SUNY-SB).
4) Recruited ORISE post doc to be funded by the National Toxicology Program (NTP).

FY 2001 Plans:

1) Develop computational procedures to analyze phototoxicity data.
2) Conduct Monte Carlo simulation study to evaluate new procedures relative to traditional testing approaches.
3) Develop manuscript on testing for dose-related trend.
4) Analyze data under the CRADA.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Interagency Agreement on Developing and Evaluating Risk Assessment Models for Key Waterborne and Foodborne Pathogens and Chemicals***	***P00422***	***None***	***Predictive Toxicology***

Objective(s):

To develop and to evaluate risk assessment models and chemical risk assessments for food and water. This is a proposal for a new interagency agreement between NCTR and EPA's National Center for Environmental Assessment.

FY 2000 Accomplishments:

1) Set up an IAG with EPA's National Center for Environmental Assessment to conduct collaborative research on microbial risk assessment and on risk assessment for chemical mixtures.
2) FY 2000 funding from EPA.
3) Drafted a protocol to conduct animal experiments on model development for transmission kinetics of infection by *Cryptosporidium parvum*, a foodborne pathogen of critical concern to FDA.
4) Began development of a strategy for examining various strategies for assessing the cumulative risk of mixtures of chemicals, in particular those having similar modes of action.

FY 2001 Plans:

1) Finalize initial animal protocol in conjunction with NCTR, CFSAN, and EPA.
2) Conduct animal experiments on *C. parvum*.
3) Carry out cumulative risk assessment for selected organophosphates on EPA's Candidate Contaminant List with EPA scientists.
4) Develop a protocol for a refined strategy for cumulative risk assessment of mixtures.
5) Report results to EPA and FDA.
6) Prepare manuscripts for publication.

◆ ***Risk Assessment (General)*** — ***S00116*** — ***None*** — ***Concept-Driven***

Objective(s):

Efforts in the improvement of Risk Assessment.

FY 2000 Accomplishments:

1) Continued to work on change-point dose-response models for continuous data.
2) Continued to work on dose-response models for clustered exchangeable binary data.
3) Completed work on combining uncertainty factors when setting exposure levels. Presented talk at FDA Science Forum and had one paper published.
4) Initiated work on risk-based Rank Fish Detector (RFD).
5) Submitted manuscript for publication.
6) Continued work on method for body-weight-adjusted analysis of tumor data. Submitted manuscript for publication.

7) Finalized work on dose-response modeling for mixed populations and for developmental neurotoxicity.
8) Two papers published.

FY 2001 Plans:

Continue risk assessment research efforts.

- ***Modification and Application of Quantitative Risk Assessment Techniques to FDA-regulated Products*** — ***S00174*** — ***CDER, CDRH, CFSAN, CVM*** — ***Method-Driven***

Objective(s):

In response to requests from scientists and regulators at CDRH, CDER, CFSAN, and CVM, using available toxicological data, conduct cancer and noncancer risk assessments of FDA-regulated products to assist in establishing "safest" conditions of exposure to toxic substances.

FY 2000 Accomplishments:

1) Developed a biologically based dose-response model for predicting the risk of mouse liver tumors as a result of FB_1 exposure. Integrated data on tissue size, cell proliferation and sphingolipid metabolism.
2) Successfully predicted liver tumor risk for female and male mice.
3) Presented one talk and two posters at the FDA Workshop on Fumonisin Risk Assessment.
4) Two manuscripts accepted for publication.

FY 2001 Plans:

Re-initiate collaboration with CFSAN colleagues to explore extending the threshold-of-regulation concept, now applied to indirect food additives (e.g., contact materials from packaging, etc.), to direct flavor additives.

- ***Application of Biometrical Procedures for NTP Projects*** — ***S00175*** — ***None*** — ***Concept-Driven***

Objective(s):

In response to requests from NCTR scientists, modify and/or apply statistical techniques to the design, conduct, analysis, and interpretation of NTP studies to identify and assess the cancer and noncancer risks of potentially toxic substances.

FY 2000 Accomplishments:

1) Assisted principal investigators and contract statisticians in determining most appropriate statistical analyses to perform on various NTP studies.
2) Assisted principal investigators and contract experimental liaisons with the experimental design and allocation of animals for various NTP studies.

FY 2001 Plans:

Continue to provide statistical support for NTP projects on an *ad hoc* basis.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Turturro, Angelo

- ***$B_6C_3F_1$ (Hybrid-NTP) Mouse Fed NIH31 Diet*** — ***E0050300*** — ***None*** — ***Concept-Driven***

Objective(s):

FY 2000 Accomplishments:

1) Compared the effects of caloric restriction on agent-induced increases in mutagenicity.
2) Presented the above results to the Society of Toxicology (won a prize for one of the best presentations in risk assessment) in March 2000.
3) Contributed efforts based on work with analysis of effect of body weight on toxicity, leading to two newsletter articles and two book chapters.

FY 2001 Plans:

Will expand mutagenicity model into a paper investigating effects on carcinogenicity using standard models, and continue efforts in elaborating body weight effects.

- ***C57Bl6/NNIA Mouse Fed NIH31 Diet*** — ***E0050400*** — ***None*** — ***Concept-Driven***

Objective(s):

FY 2000 Accomplishments:

Asked to provide work based on results of this and other caloric restriction experiments, resulting in two publications.

FY 2001 Plans:

Submit a manuscript on the effects of caloric restriction on both pre-neoplastic and neoplastic lesions in these mice.

- ***Development of a Model for the Transmission Kinetics of Infection by Cryptosporidium parvum with Acquistion of Data on Key Parameters*** — ***E0708201*** — ***EPA*** — ***Concept-Driven***

Objective(s):

1) To standardize the virulence of doses of *Cryptosporidium parvum* used in this and subsequent studies.
2) To investigate the suitability of the Brown-Norway rat as a model for *Cryptosporidium parvum* infectivity in humans or the C57Bl/6 mouse chemically suppressed with dexamethasone if BN is unsuitable.
3) To compare *Cryptosporidium parvum* infectivity for model animals with age and pegnancy which may influence immunocompetence.
4) To compare *Cryptosporidium parvum* infectivity for model animals with chemical immunosuppression other than by dexamethasone.
5) To compare *Cryptosporidium parvum* infectivity in animals with immunosupression models similar to the effects of AIDS.

6) To compare *Cryptosporidium parvum* infectivity in animals with physiological stress and nutritional immunosupresssion models.
7) To use these data in pathogen virulence and host susceptibility in a model for the transmission dynamics of *Cryptosporidium parvum* in human outbreaks.

FY 2000 Accomplishments:

1) Prepared a protocol to develop information for use in risk assessment models of microbial pathogens.
2) Worked with models of pathogen secondary transmission.

FY 2001 Plans:

1) Present interim results to EPA.
2) Work on model.
3) Start animal work.

PI: Young, John

- ***Computational Predictive System for Rodent Organ-Specific Carcinogenicity*** — ***E0708301*** — ***CFSAN*** — ***Predictive Toxicology***

Objective(s):

Using modern SAR technology and statistical approaches, an expert system can be developed to predict rodent carcinogenicity.

FY 2000 Accomplishments:

Developed a concept paper.

FY 2001 Plans:

1) Develop and implement research protocol.
2) Interact with CFSAN.
3) Present paper on method at the Computational Intelligence Methods and Application (CIMA) meeting, June 2001, Bangor, Wales.

- ***Species Comparison Utilizing a PBPK Model*** — ***P00393*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Pharmacokinetic data from the literature will be excerpted and adapted to be simulated via a PBPK model. Initially the literature data will be limited to dexamethasone, cocaine, and methyl mercury. Species comparisons will be made utilizing this single pharmacokinetic model.

FY 2000 Accomplishments:

1) Two manuscripts have been prepared from this work for publication.
2) The methyl mercury database has been completed and was partially analyzed using the rough set methodology.
3) The dexamethasone database has been constructed.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) Continue analysis of the methyl mercury database.
2) Start analysis of the dexamethasone database.
3) Initiate construction of the cocaine database.

◆ ***Methadone Pharmacokinetics*** ***P00420*** ***None*** ***Agent-Driven***

Objective(s):

All 30 data sets from the methadone rat study (E0695701) have been simulated on the hybrid computer system using a two-compartment model. These same data sets have been fit using NONMEM, the nonlinear mixed-effects modeling program from the University of California. Bugs still remain in the adaptation of the NONMEM program to the specific application in this experiment. The hours requested on this project are to finish analyzing the datasets and to write a manuscript comparing the two methods of analysis.

FY 2000 Accomplishments:

This project was reactivated from E0695701 to P00420 for manuscript preparation.

FY 2001 Plans:

Write manuscript.

PI: Zheng, Qi

◆ ***Combining Carcinogenesis Models with Pharmacokinetic Models*** ***E0703001*** ***None*** ***Method-Driven***

Objective(s):

1) To explore methods for using physiologically based pharmacokinetic models as tools for allowing target dose to be directly incorporated into stochastic carcinogenesis models, and hence improve risk assessment for various kinds of carcinogenic chemicals.
2) Within the context of using combined models, to investigate the feasibility of estimating certain biological parameters from data, if such parameter values are not readily available in the literature.

FY 2000 Accomplishments:

1) Conducted a comprehensive survey of the computational aspects of fluctuation analysis which is the oldest and the most sophisticated approach for measuring mutation rate. Results from this investigation have led to a manuscript.
2) Presented the above results to a joint meeting of the Genetics Society of Canada and the Genetics Society of America, in Vancouver, June 2000.

FY 2001 Plans:

1) Assemble the accumulated computer code into a useful package for tutorial and real data analysis purposes.
2) Explore the possibility of using genomics to solve carcinogenesis problems.

FY 2000 Publications

Chang, J.Y., Ahn, H. and Chen, J.J., On Sequential Closed Testing Procedures for a Comparison of Dose Groups with a Control, Communications in Statistics. Accepted: 11/29/1999 **(E0700901)**.

Chang, J.Y., Ahn, H. and Chen, J.J., Order-Restricted Dose-Related Trend Tests, Statistics in Medicine. Accepted: 7/21/2000 **(E0700901)**.

Chen, J.J., Lin, K.K. and Arani, R.B., Weighted P-Value Adjustments for Animal Carcinogenicity Trend Tests, Biometrics, 56:171-176. Accepted: 11/18/1999 **(E0700901)**.

Chen, J.J., Reproductive/Developmental Studies, Encyclopedia of Biopharmaceutical Statistics, 445-452. Accepted: 11/24/1999 **(S00116)**.

Chen, J.J., Tsong, Y. and Kang, S., Tests for Equivalence Between Two Proportions, Drug Information Journal. Accepted: 10/27/1999 **(E0706201)**.

Delongchamp, R.R., Young, J.F., Tissue Sphinganine as a Biomarker of Fumonisin-Induced Apoptosis, Food Additives and Contaminants. Accepted: 5/4/2000 **(S00174)**.

Frame, L.T., Ozawa, S., Nowell, S.A., Chou, H., Delongchamp, R.R., Lang, N.P. and Kadlubar, F.F., A Simple Colorimetric Assay for Phenotyping the Major Human Thermostable Phenol Sulfotransferase (SULT1A1) Using Platelet Cytosols, Drug Metabolism and Disposition, 28:1063-1068. Accepted: 6/1/2000 **(NA)**.

Freni, S., Lewis, S.M., Mayhugh, M.A., Jairaj, K., Arani, R.B., Turturro, A., McCabe, B.J. and Hart, R.W., Improved Equations for Estimating the Resting Metabolic Rate, Human and Ecological Risk Assessment, 6(6):1039-1054. Accepted: 9/15/2000 **(E0695401)**.

Gaylor, D.W., Kodell, R.L. and Chen, J.J., Human Cancer Risk Estimates Are Reduced When Based on Relative Risk for Common Rodent Tumors, Regulatory Toxicology and Pharmacology, 30 (3):283-284. Accepted: 12/15/1999 **(S00116)**.

Hansen, D.K., Laborde, J.B., Wall, K.S., Hinson, W.G., Pipkin, J.L., Shaddock, J.G., Lyn-Cook, L.E. and Young, J.F., Dose-Response of Retinoic Acid Induced Stress Protein Synthesis and Teratogenesis in Mice, Reproductive Toxicology, 15(1):31-41. Accepted: 9/22/2000 **(E0697601)**.

Hashemi, R.R., Tyler, A.A., Epperson, C. and Young, J.F., Knowledge Discovery from Sparse Pharmacokinetic Data, Proceedings of SAC 2000 - 15th ACM Symposium in Applied Computing. Accepted: 1/20/2000 **(P00393)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Hinson, W.G., Pipkin, J.L., Wu, J. and Young, J.F., Using Visualization to Confirm Mathematical Analysis, Proceedings of the 3rd International Workshop on Intelligent Control and Systems, Atlantic City, NJ. Accepted: 2/1/2000 **(NA)**.

Hinson, W.G., Quantitative Analysis of Nuclear Protein Changes in Cells Exposed to Magnetic Fields. Proceedings of the WAC 2000, Maui, Hawaii, Proceedings of the WAC 2000, Maui, Hawaii. Accepted: 6/1/2000 **(NA)**.

Hsueh, H., Liu, J. and Chen, J.J., Unconditional Exact Tests for Equivalence or Non-inferiority for Paired Binary Endpoints, Biometrics. Accepted: 9/15/2000 **(E0706201)**.

Kang, S., Generating Correlated Binary Variables with Complete Specification of Joint Distribution, Biometrical Journal. Accepted: 7/4/2000 **(S00032)**.

Kang, S., Chen, J.J., An Approximate Exact Test of Non-Inferiority Between Two Proportions, Statistics in Medicine. Accepted: 11/12/1999 **(E0706201)**.

Kang, S., Kodell, R.L. and Chen, J.J., Incorporating Model Uncertainties Along with Data Uncertainties in Microbial Risk Assessment, Regulatory Toxicology and Pharmacology, 32:68-72. Accepted: 4/12/2000 **(E0704501)**.

Kodell, R.L., Lin, K.K., Thorn, B.T. and Chen, J.J., Bioassays of Shortened Duration for Drugs: Statistical Implications, Toxicological Sciences, 55 (2):415-432. Accepted: 1/13/2000 **(E0690201)**.

Kodell, R.L., Risk Assessment Methods for Determining Spacecraft Water Exposure Guidelines, Methods for developing spacecraft water exposure guidelines, :75-107. Accepted: 5/17/2000 **(S00032)**.

Kodell, R.L., Young, J.F., Delongchamp, R.R., Turturro, A., Chen, J.J., Gaylor, D.W., Howard, P. and Zheng, Q., A Mechanistic Approach to Modeling the Risk of Liver Tumors in Mice Exposed to Fumonisin B_1 in the Diet, Food Additives and Contaminants. Accepted: 5/2/2000 **(S00174)**.

Poehlman, E., Turturro, A., Bodkin, N., Cefalu, W., Heymsfield, S., Holloszy, J., Kemnitz, J. and McCarter, R., Caloric Restriction Mimetics: Physical Activity and Body Composition Changes, Journal of Gerontology. Accepted: 9/1/2000 **(E0699801)**.

Razzaghi, M. and Kodell, R.L., Dose-Response Modeling for Developmental Neurotoxicity Data, Environmental and Ecological Statistics, 7:191-203. Accepted: 2/18/2000 **(S00116)**.

Turturro, A., Hass, B.S. and Hart, R.W., Does Caloric Restriction Induce Hormesis?, Human and Ecological Risk Assessment, 19:320-329. Accepted: 1/1/2000 **(E0050300)**.

Wolf, N.S., Li, Y.S., Pendergrass, W., Schmieder, C.R. and Turturro, A., Normal Mouse and Rat Strains as Models for Age-related Cataract and the Effect of Caloric Restriction on its Development, Experimental Eye Research, 70:683-692. Accepted: 2/7/2000 **(E0050301)**.

Yang, M., Delongchamp, R.R. and Ozawa, S., Relationship Between NAT1 Genotype and Phenotype in a Japanese Population, Pharmacogenetics, 10(3):225-232. Accepted: 2/23/2000 **(NA)**.

Young, J.F. and Luecke, R., Interspecies Pharmacokinetic Model Validation and Allometry, Proceedings of the 3rd MathMod Vienna Conference, 2:567-570. Accepted: 2/2/2000 **(P00393)**.

Young, J.F., Gough, B.J., Suber, R.L. and Gaylor, D.W., Correlation of Blood Cholinesterase Levels with Toxicity of Sarin in Rats, Journal of Toxicology and Environmental Health, Part A, 62(3):161-174. Accepted: 8/4/2000 **(E0624700)**.

CHEMISTRY

Director: ***Robert J. Turesky, Ph.D.***
Telephone: **870-543-7301**
Toll Free: **800-638-3321**
E-mail: **rturesky@nctr.fda.gov**

Dr. Shannon Snellings working on the FreshTag™ Experiment.

Executive Summary

The Division of Chemistry significantly contributes to NCTR/FDA-directed research initiatives through both its analytical support services and a diverse range of innovative research programs. The Division provides expertise in many areas of chemistry including analytical and synthetic organic chemistry, the development of instrumentation and sensor technology for rapid screening, Nuclear Magnetic Resonance (NMR) and mass spectrometry, computational chemistry, artificial intelligence and the application of analytical biochemical approaches to the solution of toxicological questions; all of these expertises are used in a Divisional effort to advance the FDA's goals and research initiatives.

There have been several changes in personnel over the past year including the recruitment of a new Division director, Robert J. Turesky, Ph.D., who provides expertise in bioanalytical and chemical research techniques. These skills will be applied to research programs involving interspecies extrapolation and risk assessment of dietary contaminants, and the assessment of pro- and antioxidant status that may affect human health.

The Division has contributed to interagency collaborations such as the National Toxicology Program (NTP)/National Institute of Environmental Health Sciences (NIEHS). Acute and/or chronic experiments for chloral hydrate, ethinyl estradiol, genistein, leucomalachite green, malachite green, methoxychlor, nonylphenol, urethane and vinclozolin were supported during 2000. The chronic bioassay of chloral hydrate in male $B_6C_3F_1$ mice using idealized body weight curves that are normalized to modulation of caloric intake (Project E211701) has been completed. Chloral hydrate is a hepatocarcinogen and induces several enzymes that are markers of peroxisome proliferations, providing a mechanistic basis for the mode of action for chloral hydrate carcinogenicity. A Good Laboratory Practice (GLP) report and manuscripts describing the findings are under preparation. During 2001, the Division will continue to support the NTP/NIEHS Interagency Agreement (IAG) experiments for ethinyl estradiol, genistein, leucomalachite green, malachite green and also develop methods of analyses to support planned *Aloe vera* experiments.

In support of initiatives from the Center for Veterinary Medicine (CVM), an analytical method has been established for quantification of 14 sulfonamide drug residues in fish tissues using high performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection (Projects E700601 and P00415). During the year 2001, incurred dosed tissues will be evaluated for final validation of the method. An analytical HPLC technique also was established to quantitate incurred amoxicillin and lincomycin residues in catfish with analyte identity confirmed by mass spectroscopy (Project E0693601). A rapid method for the measurement of erythromycin in salmon by HPLC-electrochemical detection (ECD) and HPLC-Mass Spectrometry (MS) will also be established during 2001 (Project E0698001). Collaboration with the Center for Food Safety and Applied Nutrition (CFSAN) was initiated on identification of pharmacologically active constituents in herbal medicines and dietary supplements. Improved extraction methods including supercritical fluid and ultrasonic techniques were developed to recover biologically active components, including hyperforin and hypericin in St. John's Wort, which is used as a "folk remedy" for a number of different illnesses (Project 705601). These investigations will be extended to analyses of components in Echinacea. A research initiative with the Office of Women's Health has been established for 2001 (X0031) to assess the impact of dietary supplements and constituents such as hyperforin on modulation of several enzymes involved in estrogen metabolism, including cytochrome P450 and UDP-glucuronosyl transferases.

In the area of computational chemistry and structure-activity relationships, a novel approach has been developed using patent-pending Spectral Data-Activity Relationships (SDARs) to predict biological, chemical, and physical properties of molecules (Project E0706801). An international and USA patent was filed for this approach, and we have published SDAR models of estrogen receptor binding and dechlorination of chlorobenzene analogues. During 2001, collaborative advances will be made on the SDAR model, including the production of a SAR-SDAR hybrid model, a cancer prediction model, and a neural network model. The use of SDAR, quantum mechanics and statistical/AI techniques to the prediction of total dioxin equivalent factors for dioxins, PCBs and furans will be undertaken in 2001, with the hope of establishing toxic equivalent factors, which may be applied to the risk assessment of foods containing these chemicals expressed as toxic dioxin equivalents.

NMR techniques have also been applied to estimate drug purity (Project E0693801), where contamination levels of less than 0.1% can be detected. The benefit of this approach is that unknown impurities may be rapidly detected. The hope is that NMR spectroscopy can be used as a rapid tool to screen for product adulteration, protecting the consumer against contaminated medicines. This approach will be continued in 2001 for validation.

The mass spectrometry group has continued important collaborative research with the Division of Microbiology on microbial metabolism of various drugs and feed additives (Projects E06901, E07007, E07052, and E06942), resulting in several manuscripts. Mass spectrometric methods have been developed with potential applications related to the safety of foods, seafood and to combat bioterrorism. This includes methods for the

identification of potentially toxic *Vibrio* bacteria, where several different strains were characterized by pyrolysis/mass spectrometry (MS) and Matrix Assisted Laser Desorption-Mass Spectrometry (MALDI/MS) techniques. Matrix Assisted Laser Desorption/Time of Flight-Mass Spectrometry (MALDI/TOF-MS) (Projects E0700501, E0693101, X10009) was used to demonstrate the potential for the rapid detection of virulence-related proteins from bacteria using samples of contaminated lettuce, water and cotton. The absence of time-intensive, preanalysis culture-steps in these analyses suggests that this approach also may be useful for applications in bioterrorism. In the year 2001, Fourier transform mass spectrometry will be applied for characterization of bacteria in collaboration with University of Arkansas at Fayetteville. The application of a novel and rapid pyrolysis/metastable atom bombardment (MAB)/MS for characteristic fingerprints in chemotaxonomic characterization of bacteria without the need for culture steps was undertaken and will continue during 2001 in collaboration with the University of Montreal and University of Arkansas at Little Rock (UALR) (Project E0693101). In addition, the mass spectrometry group plans to implement protein characterization capabilities to support research initiatives in proteomics at NCTR.

Chemical sensor technology (FreshTag™) for the assessment of food quality has also been further developed during 2000 (Project E0687401), and the concept has evolved into both a commercial version and a consumer version. Testing by the National Oceanic and Atmospheric Administration (NOAA) demonstrates that this approach has much promise. This work will be extended during the year 2001 to detect other endpoints that are measures of product quality and freshness, and include indoles and aldehydes. As an extension of this work, an interagency agreement has been established with the Federal Aviation Administration (FAA) to develop rapid sensor detection methods to screen for explosives to protect the air transportation industry (Project E0708101).

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Ang, Catharina

- ***Development of Analytical Methods for Determination of Amoxicillin and Lincomycin in Fish Tissues*** — ***E0693601*** — ***None*** — ***Method-Driven***

Objective(s):

1) To develop highly sensitive analytical methods utilizing reversed-phase HPLC or gas chromatography (GC) for determining trace levels of amoxicillin and lincomycin residues in fish tissues. Specifically, the goal is to develop analytical methods which can be applied to determine amoxicillin in catfish muscle tissue and salmon muscle and skin tissues at 10 parts per billion (ppb) and to determine lincomycin in salmon muscle and skin tissues at 100 ppb as suggested by the FDA Center for Veterinary Medicine (CVM).
2) Separate procedures/solvent systems for the extraction, cleanup, and HPLC analysis of each antibiotic are expected to be necessary because of the structural differences between amoxicillin and lincomycin. However, analytical residues in both the catfish and salmon tissue substrates will be developed if feasible.

FY 2000 Accomplishments:

Manuscript submitted on LC analysis with MS confirmation of Amoxicillin in Catfish.

FY 2001 Plans:

The remaining phase of this project and E0693611 have been transferred to P00423. The final phase of this study is the inter-laboratory collaboration for validation of the method. The collaboration will be sponsored by the Center for Veterinary Medicine (CVM). It is anticipated that the inter-laboratory studies will produce one manuscript.

- ***Antimicrobial Effect, Chemical and Biological Characterization of Selected Medicinal Botanical Products.*** — ***E0705601*** — ***CFSAN*** — ***Method-and Agent-Driven***

Objective(s):

1) To investigate chemical, biological and anti-microbiological activities of selected botanical products including St. John's Wort (SJW), ginkgo biloba (GB) and goldenseal (GS).
2) To develop analytical methodologies for the determination of bioactive components of SJW, GB, GS, and aristolochia.
3) To provide needed information in addressing safety issues of botanical dietary supplements in conjunction with future toxicological studies involving these medicinal herbs, to aid the Agency in regulatory and labeling decisions.

4) To develop alternative means using these products for the reduction of microbial contamination of our food supply.

FY 2000 Accomplishments:

1) Two manuscripts related to this work have been published: Evaluation of active compounds in St. John's Wort dietary supplement by LC/PDA and MS and Optimization of extraction conditions for *Hypericum perforatum* using response surface methodology.
2) Additional data have been obtained relative to supercritical fluid extraction, ultrasonic techniques and improved HPLC methods for the determination of ten compounds in St. John's Wort plant and dietary supplements.
3) An addendum of the project (E0705611) was completed with objectives in the development of analytical methodologies for herbal bioactive components in functional food systems.

FY 2001 Plans:

The remaining phase of this project (E0705601) will focus on the addendum (E0705611) which is in collaboration with the Center for Food Safety and Applied Nutrition (CFSAN) and financially supported by the collaborator. Major research activities will be the development of analytical methodologies for bioactive compounds hyperforin and hypericin, pseudohypericin, and adhyperforin in various functional foods containing fortified St. John's Wort and caftaric acid, isobutylamides, echinocoside, and cichoric acid which are present in echinacea.

◆ ***Determination of Amoxicillin and Lincomycin Incurred Residues in Salmon and Tilapia for Selection of FDA Method Trial Study Materials*** ***P00423*** ***CVM*** ***Method-Driven***

Objective(s):

Amoxicillin and lincomycin have been identified as having toxicological significance by the CVM, NCTR, and the U.S. Department of Agriculture (USDA) and have been scheduled for incurred-residue FDA method trials. Methods of analysis have been developed and validated for amoxicillin in catfish and salmon and lincomycin in salmon.

1) Validation of the above methods for incurred residues of amoxicillin in salmon and tilapia and lincomycin in salmon are needed.
2) Documentation of incurred-residue confirmation methods by Liquid Chromatography/Mass Spectrometry (LC/MS) are also needed for these regulatory methods.

FY 2000 Accomplishments:

One manuscript related to this work has been published under E0693611.

FY 2001 Plans:

The final phases of E0693601 and E0693611 (methods development) represent the inter-laboratory collaboration to evaluate the ruggedness of these analytic techniques.

Title	Project Number	Collaborator	Strategic Research Goal

The method trial is to be organized by the CVM.

◆ ***Chemical Differences and Toxicological Effects of Dang qui (Angelical sinensis) Root Preparations*** — ***X10016*** — ***None*** — ***Agent-Driven***

Objective(s):

FY 2000 Accomplishments:

Angelical sinensis is widely prescribed by herbalists in Asian countries, however it is widely available as over-the-counter preparations in the United States. This project is under internal review. A portion of this proposal involves a pilot study with human subjects (ten female participants).

FY 2001 Plans:

1) The project proposal will be completed and evaluated, including Human Subjects approval. A clarification of the ability of participation by FDA employees is being sought.
2) Preliminary analytical techniques found in the literature will be evaluated for their utility in the project.
3) Additional methodology (e.g., hormonal status) will also be established for the pilot study. Ten potential participants will be recruited.

PI: Beger, Richard

◆ ***Producing Spectrometric Data Activity Relationship (SDAR) Models for Compounds Binding to Receptors of Toxic Responses.*** — ***E0706801*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To produce Spectrometric Data-Activity Relationship (SDAR) models using ^{13}C NMR and electron impact (EI)/MS data to predict the potential binding affinity of compounds to specific receptors or produce toxicological response. The major benefit of the experimental SDAR approach is its flexibility since the spectral data can be used for other toxicological systems. (May require some refinement.)

FY 2000 Accomplishments:

1) Three manuscripts were published.
2) Three additional manuscripts of the SDAR process have been sent out for publication.

FY 2001 Plans:

1) Partnerships will be sought to expand these techniques to other biological endpoints.
2) Preliminary contacts related to carcinogenicity have been initiated with the Division of Biometry and Risk Assessment.
3) A task order has been written to examine the potential of the QSAR/SDAR hybrid to predict protein binding.

Title	Project Number	Collaborator	Strategic Research Goal
◆ *NMR of DNA and Adducts*	*X90005*	*None*	*Method-Driven*

Objective(s):

FY 2000 Accomplishments:

Initiatives in this proposal have been confined to travel to St. Jude Children's Hospital. The principal investigator is a consultant for experimental design/data interpretation from the 800 and 600MHz NMR equipment.

FY 2001 Plans:

These experiments are subject to acquisition of an enhanced NMR capability.

PI: Billedeau, Stanley

◆ ***Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography***	*E0698001*	*None*	*Method-Driven*

Objective(s):

To develop determinative and confirmatory analytical chemical procedures using high performance liquid chromatography/electrochemical detection and high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric detection for erythromycin A in biological samples taken from agricultural animals. Specifically, the goal is to develop complete methods for the analysis of Erythromycin A in muscle and liver tissue from poultry, non-processed bovine milk, and muscle tissues from salmon, catfish and shrimp. Sensitivity levels for these methods are expected to be at least 100 parts per billion for liver tissue and 50 parts per billion for muscle tissue and milk as requested by the Center for Veterinary Medicine (CVM).

FY 2000 Accomplishments:

1) This project has been reactivated and assigned a new principal investigator and collaborating chemists; the CVM has reiterated their interest in this method.
2) Initial work has been to organize and evaluate existing records for progress.
3) The initial cleanup procedures proved efficient, however, the extraction requires refinement to optimize the application for catfish.

FY 2001 Plans:

^{14}C-Erythromycin has been obtained to facilitate effective cleanup procedures. It is anticipated that HPLC/ECD will provide optimum detection for the routine assay and that a published HPLC/MS method can be used for confirmation analysis.

◆ ***Development of Methods for Analysis of Volatile and Nonvolatile N-nitrosamines in Relevant Cosmetics and Nitrite Cured Meat***	*E0698901*	*None*	*Method-Driven*

Title	Project Number	Collaborator	Strategic Research Goal

Products

Objective(s):

1) To develop methods for extraction, cleanup, and analysis of non-volatile N-nitrosamines in cosmetics and meat products using combined liquid chromatography (LC) detection methods with confirmation by compatible mass spectrometry (MS) ionization methods.
2) To investigate the applicability of Liquid Chromatography-electron spray ionization/mass sprectroscopy (LC-ESI/MS) and/or (LC-APCI/MS) as a multi-residue, trace-level, quantitative technique for analysis of volatile, semi-volatile, and non-volatile N-nitrosamines in these consumer products.

FY 2000 Accomplishments:

1) The results of this project have been reported at the 48th American Society of Mass Spectrometry (ASMS) conference.
2) The manuscript has been submitted for publication.

FY 2001 Plans:

This project has ended.

PI: Evans, Frederick

◆ ***A New Approach to the NMR Spectroscopy of Drug Purity and the Public Health Implications*** — ***E0707801*** — ***None*** — ***Concept-Driven***

Objective(s):

1) To determine properties and optimize conditions of the NMR spectrometer at the NCTR under high,dynamic-range conditions.
2) To develop concepts and methodology for application of NMR spectroscopy to the investigation of very-low-level impurities in drugs using results on genistein as a model.

FY 2000 Accomplishments:

A draft of this proposal has completed internal review and was submitted to the Center for Drug Evaluation and Research (CDER) NMR spectroscopists for evaluation. It was concluded that the research is sound and could advance the utility of NMR spectroscopy to evaluate impurities at a low level (<0.1%). It was also noted that software upgrades to NCTR systems would be required to advance this project efficiently.

FY 2001 Plans:

This proposal should enhance detection and measurement of otherwise unknown impurities in drug candidate substances which could lead to faster drug development and reduced medical side-effects in some cases. It could lead to the approval of drugs that would otherwise be rejected as unsafe, leading to an even safer drug supply. The current study is a first step in this direction. This proposal represents advancement in

technique that could be used to characterize impurities in drug lots that may originate from non-licensed manufacturers (i.e., economic fraud). When this method is developed, it will provide a relatively sensitive and rapid technique for detecting/characterizing impurities. In other applications this technique may represent a tool in rapid response to suspected contamination or illegal manufacture of a drug.

PI: Gehring, Theresa

- ***Development of Multiresidue Methods to Determine and Confirm Sulfonamides in Edible Tissues of Aquacultured Species*** — ***E0700601*** — ***None*** — ***Method-Driven***

Objective(s):

To develop analytical chemical methods to determine and confirm sulfonamide (SA) residues at the 1-10 ng/g level in edible tissues of aquacultured species. Technologies used will include liquid chromatography (LC) with postcolumn derivatization and fluorescence detection for the determinative procedure and liquid chromatography with atmospheric pressure chemical ionization mass spectrometry LC-APCI/MS for the confirmatory procedure.

FY 2000 Accomplishments:

The inter-laboratory methods trial portion of this study was assigned to P00415.

FY 2001 Plans:

1) The certification of ranges for the inter-laboratory trial will be completed as required.
2) The final report for the methods trial will be prepared if the collaborative studies are completed this year.

- ***Determination of Sulfonamide Incurred Residues in Catfish and Shrimp for Selection of FDA Method Trial Study Materials*** — ***P00415*** — ***CVM*** — ***Method-Driven***

Objective(s):

CVM/USDA/NCTR have identified six sulfonamides of toxicological significance to be included in an incurred-residue FDA method trial. Methods of analysis have been developed and validated; all included sulfas in catfish and shrimp.

FY 2000 Accomplishments:

The incurred-residue methods trial studies for shrimp/catfish remain unfinished. The study, comprised of four sulfonamides, each at two levels, in each species is 2/3 complete. The incurred-residue level for the target ranges for shrimp and catfish that were prepared by the University of Arizona and Stuttgart (AR) National Aquaculture Research Center (SNARC) did not meet the specified ranges of the trial delaying completion; consequently, they will be prepared again. The assay-developing laboratory, NCTR, validates the certification of ranges for the inter-laboratory trial.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) The certification of ranges for the inter-laboratory trial will be completed as required.
2) The final report for the methods trial will be prepared provided the collaborative studies are completed this year.

PI: Lay, Jackson

◆ ***Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS).*** *E0700501* *CFSAN* *Method-Driven*

Objective(s):

1) To evaluate the potential use of matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a method for the rapid identification of whole bacteria, either by comparison with archived reference spectra or by co-analysis with cultures of known bacteria.
2) To establish a standard set of conditions for the acquisition of MALDI/TOF mass spectra from bacteria suitable for use in bacterial identification.
3) To obtain some measure of the distribution of signals (ions at specific masses) obtained using standard MALDI/TOF MS conditions based on the analysis of a variety of related and unrelated bacteria.
4) To use standard (pattern recognition) as well as newer (artificial intelligence and principal components analysis) mass spectral recognition techniques to evaluate whether or not the standardized mass spectra obtained from bacteria are sufficiently distinct to allow identification of specific bacteria or to select related bacteria from a group.
5) To evaluate the use of mass spectral recognition techniques for the identification of bacteria from mixtures based on MALDI/TOF MS analysis of the mixture.
6) To determine the minimum number of bacteria necessary for obtaining standard mass spectra.
7) To evaluate the effects on the reproducibility of spectra obtained from whole bacteria under different conditions of sample handling, storage, and cell growth.

FY 2000 Accomplishments:

1) Demonstrated gene-specific, marker detection (acid resistance) across bacterial strains and characterization of bacteria from natural substrates without prior enrichment.
2) This work has resulted in three invited reviews and two manuscripts this year.
3) An invited collaboration to the CFSAN for development of methods of *Vibrio* sub-speciation on MALDI TOF mass spectrometers.

4) A joint FDA/International Association of Official Analytical Chemists (IAOAC) symposium was established to discuss the role of mass spectrometry in the rapid speciation of bacteria (June 2001).
5) Additional collaborative efforts (analysis of fluctuating body weights for predicting tumor outcomes) utilizing computational methods were submitted for publication.

FY 2001 Plans:

Future work on these methods are in draft protocol development comparing MALDI TOF mass spectrometry and MALD FT mass spectrometry (see X10009).

◆ ***Biomarkers for Virulence of Listeria by Mass Spectrometry*** — ***X00024*** — ***None*** — ***Method-Driven***

Objective(s):

FY 2000 Accomplishments:

Strain-specific fingerprints were detected for several species of *Vibrio* bacteria, including *V.p.* and *V.v*., but at that time the spectral patterns specific for the most toxic strains of *V.v*. from seafoods were not elucidated adequately for any regulatory use of the existing data.

FY 2001 Plans:

The original concept with *Listeria sp*. will be consolidated with the other bacterial studies that will be conducted in collaboration with spectroscopists located at CFSAN and the University of Arkansas at Fayetteville. (re: X10009).

◆ ***Comparison of MALDI TOF MS and MALDI FTMS for Typing Vibrio Bacteria*** — ***X10009*** — ***None*** — ***Predictive Toxicology***

Objective(s):

FY 2000 Accomplishments:

The principal investigator developed the pilot studies for this proposal by transporting mass spectrometry combined with pattern recognition technology to the CFSAN:
1) The biology of learning sets is required to develop a test system.
2) Simple membrane-fractionation techniques will enhance numbers of identified proteins.
3) Sub-typing of *Vibrio parahemolyticus* is transportable to other systems.
4) The method can sub-type pathogenic from non-pathogenic *E. coli* strains.
5) A joint FDA/IAOAC symposium to discuss the role of mass spectrometry in the rapid speciation of bacteria (June 2001).

FY 2001 Plans:

Complete development of collaborative studies among personnel and equipment located at CFSAN, NCTR and the University of Arkansas at Fayetteville, AR:
1) Evaluation of transport of technology to simpler TOF instruments.
2) Evaluation of transport of technology to MALDI utilizing FT (Fourier Transform) enabling high-resolution detection in larger molecular weight ranges. It is

anticipated that one two-week assignment each quarter to CFSAN and preliminary experiments at the University of Arkansas at Fayetteville will enable these experiments.

PI: Leakey, Julian

- ***Chronic Bioassay of Chloral Hydrate in Male $B_6C_3F_1$ Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake*** — ***E0211701*** — ***CDER*** — ***Concept-Driven***

Objective(s):

1) To determine the chronic toxicity and potential carcinogenicity of chloral hydrate administered by aqueous gavage, to male $B_6C_3F_1$ mice.
2) To determine the feasibility of utilizing dietary control (i.e., the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an idealized weight curve.

FY 2000 Accomplishments:

The results of E0211701, E0211711, and E0211722 were reported to the NTP Board of Scientific Counselors in draft form. Briefly, the committee concluded that there was some evidence of hepatocarcinogenicity in the mouse and dietary control effectively controlled variables in the bioassay.

FY 2001 Plans:

1) Finalize the draft report.
2) Finalize the GLP report for all experiments (compile, finalize and file in the NCTR archive).
3) Three manuscripts will be prepared from these studies: a) Effectiveness of dietary-control for the cancer bioassay; b) Hepatocarcinogenicity of chloral hydrate; and c) Chloral Hydrate as a peroxisome proliferator.

- ***Effect of Caloric Restriction on Rat Testicular Tumor Formation*** — ***E0260201*** — ***None*** — ***Concept-Driven***

Objective(s):

All of the aims of this proposal are directed towards understanding the role of dietary components (i.e., caloric restriction) in influencing the ultimate susceptibility of the male reproductive tract to chemical insult.

FY 2000 Accomplishments:

E0260201, E0260211, and .22 are the collaborative efforts between the NCTR and the UAMS and represent the graduate thesis.

FY 2001 Plans:

Completion of the manuscripts.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Miller, Dwight

- ***Development of Devices/Methods for Determination of Food/Seafood Quality*** — ***E0687401*** — ***ORA*** — ***Method-Driven***

Objective(s):

To assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semivolatile organic compounds in spoiled seafood.

FY 2000 Accomplishments:

Resources transferred to E0699701.

FY 2001 Plans:

Final report on RFD (Rank Fish Detector) will be completed.

- ***Innovative Methods for Determining Food Quality: Decomposition, Safety and/or Economic Fraud*** — ***E0699701*** — ***ORA*** — ***Method-Driven***

Objective(s):

1) To examine the total volatile bases (TVB) and putrescine (PU), cadaverine (CD) and histamine (HS) methods for potential regulatory use and validation of TVB as an indicator of decomposition.
2) To develop rapid detection methods for the determination of decomposition analytes in seafood.

FY 2000 Accomplishments:

1) Ink identified and compounded for food contact (consumer version).
2) Alternative technologies boosting production of FreshTag™ from 11K units/week to 11M units/week developed (commercial version).
3) Organoleptic-certified samples and technologies combined into test kits for collaborative evaluation among National Marine Fisheries, University of Florida and University of North Carolina.
4) Invited review (Analysis of Off Flavors).
5) Popular Science "Best of What's New" Award.
6) Two invited symposia lectures.

FY 2001 Plans:

Continued support for commercialization of FreshTag™; both manufacturing techniques and application to regulatory decision making.

- ***Rapid Screening Test for Food Quality*** — ***E0708001*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To develop simple field-compatible methods to test for food quality.

FY 2000 Accomplishments:

Proposal submitted for peer review.

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) Complete indole instrumental method and develop colorimetric method for indole with consistent uniform color development.
2) Perfect ink deposition on plastic film using ultrasonic spray equipment.
3) Perfect isolation of lipid peroxidation methods revolving about microwave and/or headspace techniques.
4) Develop screening system for potential color-reactants for the gas-phase detection of lipid peroxidation products.
5) Develop wet chemical test for the determination of carbon monoxide in tuna.
6) Develop Ion Chromatograph method for ammonia, trimethylamine and dimethylamine in fish and shrimp to support FreshTag™.

◆ ***Application of Solid Phase Detection Systems to Explosives in Airplane Cargo*** ***E0708101*** ***None*** ***Method-Driven***

Objective(s):

1) To detect ammonia (formulation, measurement of Am concentrations around container of ammonium nitrate, reformulation of FreshTag™ chemistry for label type detection)
2) To develop PE or PVC film Shrink Rap detector.
   a) Acid detection.
   b) Detection of oxidizers such as peroxides and NO or NO_2.

FY 2000 Accomplishments:

Resources transferred through an Interagency Agreement (IAG) to NCTR for the development of solid-phase explosives detection.

FY 2001 Plans:

1) Application/extension of FreshTag™ technologies for detection of nitrogen-based explosives will be made. FreshTag™ has been shown to produce color change in the presence of ammonium nitrate, but it is not the typical acid-base color change. This is considered to be an indication that an oxidizer is possibly causing the change.
2) Develop Ion Chromatographic method to detect ammonia in headspace above ammonium nitrate and fish (E0707800). This is to prove that ammonia is or is not present.
3) Develop colorimetric detection system for gas-phase acids in headspace above explosives.
4) Develop colorimetric detection system for gas-phase oxidizers in headspace above explosives.
5) If we get positive results with either 3 or 4 above, we will develop instrumental method to prove results.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Schmitt, Thomas

- ***Development of Analytical Methods for Aloe Vera in Support of NTP/NIEHS IAG Bioassay*** — ***X00012*** — ***None*** — ***Method-Driven***

Objective(s):

FY 2000 Accomplishments:

FY 2001 Plans:

The requirements of this NIEHS/NCTR study remain unknown. The principal invesigator and/or alternates will develop the required analytical assays to support these studies. The postdoc in the Division of Biochemical Toxicology, who will direct the *Aloe vera* bioassay studies, is expected to provide design requirement guidelines between 11/01/00 and 1/01/01. The design will prescribe the analytical development required for the studies.

PI: Wilkes, Jon

- ***First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern Recognition*** — ***E0693101*** — ***CFSAN ORA*** — ***Knowledge Bases***

Objective(s):

To evaluate the feasibility of the application of pyrolysis mass spectrometry (PyMS) with computerized pattern recognition (PattRec) for the rapid identification of a sample: (a) which is a complex chemical mixture, (b) which is member of a set of such mixtures, and (c) for which there is a regulatory need to distinguish the individual members of the set. Typical examples of applications: (a) the rapid identification of culturable pathogenic and non-pathogenic bacteria in food, (b) the distinction of adulterated from unadulterated foods or cosmetics, or generic from brand-name pharmaceutical products, or (c) demonstrating the virginity of plastic materials used in food containers.

FY 2000 Accomplishments:

1) Two invited reviews of previous work *in press* and invited symposium talk *in press*.
2) Employee Invention Disclosure filed for a practical method for processing of mass spectra to determine bacterial taxonomy; a technique to track intensity variations and drift of spectra.
3) Work within the SDAR project (E0706801) also was initially completed under this project; including the final patent application.

FY 2001 Plans:

1) The patent application has been generalized to handle all types of similar data.

2) Final phases of revision will be completed with the patent attorneys and a licensee arrangement will be made.
3) Plans for further advancement of these technologies have been submitted for internal review under X10008.

- ***Universal Interface Development and Applications*** — ***E0697201*** — ***None*** — ***Method-Driven***

Objective(s):

To develop, if possible and practical, a variety of new technologies for improving high performance liquid chromatography (HPLC) detection. By eliminating hazards associated with radioactivity, it can make possible metabolic drug studies involving human subjects. Several Cooperative Research and Development Agreements (CRADAs) will be negotiated during the work to facilitate development of commercial versions of the devices which show the most promise.

FY 2000 Accomplishments:

Redesign of Model B interface performance and characterization completed and results presented jointly by NCTR and the CRADA partner at PittCon 2000. A usable Chemical Reaction Interface Mass Spectrometry (CRIMS) microwave cavity and advanced versions of diffusion cells are available for testing and re-design.

FY 2001 Plans:

Model C interface will be developed/evaluated under experiment X10010.

- ***Development of Dynamic Field Ion Mobility Mass Spectrometry*** — ***X10012*** — ***None*** — ***Predictive Toxicology***

Objective(s):

FY 2000 Accomplishments:

1) A possible post-doctoral candidate with the credentials to develop has been identified and the required H-1 visa application process has begun through the Agency.
2) A preliminary proposal has been received.

FY 2001 Plans:

1) Development of high-temperature mobility apparati and computational methods to increase the useful molecular weight range of particle detection for these instruments. It would then be useful for peptide and particle analysis including prion size molecules.
2) Development of dynamic field ion mobility mass spectrometry that would reduce instrument weight and power requirements, thus, creating a portable instrument for field applications.

Patents

D.L. Miller, R.D. Beger, J. Wilkes, J.O. Lay, Jr., and J.P. Freeman. "Methods for Predicting the Biological, Chemical and Physical Properties from their Spectral Properties". U.S. Patent No. 09/496,314 (2/2/2000).

Patent Applications Filed:

D. Miller, J. Wilkes, E. Conte, "Food Quality Indicator Device," U.S. Patent Application No. 09/116,152; Filed 7/16/1998.

Patentable Discoveries Disclosed:

J. Wilkes, F. Rafii, K. Glover, M. Holcomb, X. Cao, and J. Sutherland. "Microbial Identification Databases." The invention relates to methods of quickly identifying microorganisms, especially using databases for identifying microorganisms based upon their spectroscopic, spectrometric and chromatographic characteristics.

FY 2000 Publications

Ang, C.Y., Liu, F., Lay, J.O., Luo, W., Mckim, K.L., Gehring, T.A. and Lochmann, R., Liquid Chromatographic Analysis of Incurred Amoxicillin Residues in Catfish Muscle Following Oral Administration of the Drug, Journal of Agricultural and Food Chemistry, 48:1673-1677. Accepted: 2/11/2000 **(NA)**.

Bajic, S., Doerge, D.R., Lu, L. and Hansen, E., LC/MS Analysis of Erythromycin Using Involatile Mobile Phases With a Novel API Ion Source, Rapid Communications in Mass Spectrometry, 14:156-160. Accepted: 11/30/1999 **(NA)**.

Beger, R., Freeman, J.P., Lay, J.O., Wilkes, J.G. and Miller, D.W., Producing 13C NMR, Infrared Absorption and EI Mass Spectrometric Data Models of the Monodechlorination of Chlorobenzenes, Chlorophenols, and Chloroanilines, Journal of Chemical Information and Computer Science, 40(6):1449-1455. Accepted: 5/12/2000 **(NA)**.

Beger, R., Freeman, J.P., Lay, J.O., Wilkes, J.G. and Miller, D.W., 13C NMR and EI Mass Spectrometric Data-Activity Relationship Model of Estrogen Receptor Binding, Toxicology and Applied Pharmacology, 169:17-25. Accepted: 8/17/2000 **(E0706801)**.

Bever, R.J., Couch, L.H., Sutherland, J.B., Williams, A.J., Beger, R., Churchwell, M.I., Doerge, D.R. and Howard, P., DNA Adduct Formation by *Fusarium* Culture Extracts: Lack of Role for Fusarin C., Chemico-Biological Interactions, 128:141-157. Accepted: 8/2/2000 **(E0211101)**.

Billedeau, S.M., Holland, R.D., Heinze, T.M., Freeman, J.P., Cooper, W.M., Cooperman, A. and Lay, J.O., Analysis of Tobacco Specific N-nitrosamines (TSNAs), NNN and NNK, in Tobacco by HPLC/Particle Beam/TEA, HPLC/ESI/MS, and GC/EI/MS, Proceedings of the 48th ASMS Conference. Accepted: 8/10/2000 **(NA)**.

Doerge, D.R., Chang, C., Churchwell, M.I. and Holder, C.L., Analysis of Soy Isoflavone Conjugation *In Vitro* and in Human Blood Using LC/MS, Drug Metabolism and Disposition, 28(3):298-307. Accepted: 11/9/1999 **(NA)**.

Doerge, D.R., Chang, C., Holder, C.L. and Churchwell, M.I., Enzymatic Conjugation of the Soy Isoflavones, Genistein and Daidzein and Analysis in Human Blood Using Liquid Chromatography/Mass Spectrometry, Biochemical Pharmacology. Accepted: 11/1/1999 **(NA)**.

Guy, P.A., Gremaud, E., Richoz, J. and Turesky, R., Quantitative Analysis of Mutagenic Heterocyclic Aromatic Amines in Cooked Meat Using Liquid Chromatography - Atmospheric Pressure Chemical Ionisation Tandem Mass Spectrometry, Journal of Chromatography A, 883:89-102. Accepted: 3/17/2000 **(NA)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Hanna, G.M. and Evans, F.E., Optimization of Enantiomeric Separation for Quantitative Determination of Chiral Drug Propranolol by 1H-NMR Spectroscopy Utilizing a Chiral Solvating Agent, Journal of Pharmaceutical and Biomedical Analysis, 24:189-196. Accepted: 5/27/2000 **(NA)**.

Holland, R.D., Rafii, F., Heinze, T.M., Sutherland, J.B., Voorhees, K.J. and Lay, J.O., MALDI TOF/MS Detection of Bacterial Biomarker Proteins Isolated from Contaminated Water, Lettuce, and Cotton Cloth, Rapid Communications in Mass Spectrometry, 14:911-917. Accepted: 3/21/2000 **(E0700501)**.

Hur, H., Lay, J.O., Beger, R., Freeman, J.P. and Rafii, F., Isolation of Human Intestinal Bacteria Metabolizing the Natural Isoflavone Glycosides Daidzin and Genistin, Archives of Microbiology, 174:422-428. Accepted: 9/26/2000 **(E0700701)**.

Lay, J.O., MALDI TOF Mass Spectrometry and Bacterial Taxonomy, Trends in Analytical Chemistry, 19(8):507. Accepted: 4/17/2000 **(E0700501)**.

Liu, F., Ang, C.Y. and Springer, D., Optimization of Extraction Conditions of Active Components in Hypericum perforatum Using Response Surface Methodology, Journal of Agricultural and Food Chemistry, 48:3364-3371. Accepted: 5/24/2000 **(E0705601)**.

Liu, F., Ang, C.Y., Rankin, J.D., Beger, R., Heinze, T.M., Freeman, J.P. and Lay, J.O., Evaluation of Major Active Components in St. John's Wort Dietary Supplements by High Performance Liquid Chromatography with Photo-Diode Array Determination and Electrospray Mass Spectrometric Confirmation, Journal of Chromatography A, 888:85-92. Accepted: 4/18/2000 **(E0705601)**.

Miller, D.W., Conte, E.D., Shen, C.Y. and Perschbacher, P.W., Colorimetric Approach to Cyanobacaterial Off-Flavor Detection, Waters Science Technology, 40(6):165-169. Accepted: 12/1/1999 **(E0699701)**.

Moody, J.D., Heinze, T.M. and Cerniglia, C.E., Fungal Transformation of the Tricyclic Antidepressant Amoxapine: Identification of N-carbomethoxy Compounds Formed as Artifacts by Phosgene in Chloroform Used in the Extraction of Metabolites, Biocatalysis and Biotransformation. Accepted: 9/26/2000 **(E0690101)**.

Moody, J.D., Zhang, D., Heinze, T.M. and Cerniglia, C.E., Transformation of Amoxapine by *Cunninghamella elegans*, Applied Environmental Microbiology, 66:3646-3649. Accepted: 5/16/2000 **(E0694201)**.

Mottier, P., Parisod, V. and Turesky, R., Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromtography Coupled to Mass Spectrometry, Journal Agricultural and Food Chemistry, 48:1160-1166. Accepted: 1/25/2000 **(NA)**.

Parshikov, I., Freeman, J.P., Lay, J.O., Beger, R., Williams, A.J. and Sutherland, J.B., Microbiological Transformation of Enrofloxacin by the Fungus *Mucor ramannianus*, Applied Environmental Microbiology, 66:2664-2667. Accepted: 3/21/2000 **(E0705201)**.

Wilkes, J.G., Conte, E.D., Kim, Y., Holcomb, M., Sutherland, J.B. and Miller, D.W., Sample Preparation for the Analysis of Flavors and Off-Flavors in Foods, Journal of Chromatography A - Special Issue, 880:3-33. Accepted: 2/28/2000 **(E0693101)**.

Wilkes, J.G., Holland, R.D., Holcomb, M. and Lay, J.O., Comparison of Py-MS, MALDI-TOF/MS, and Molecular Biology Methods for Rapid Classification of *Vibrio parahaemolyticus* Outbreak Strains, Proceedings of the 48th ASMS Conference on Mass Spectrometry and Allied Topics. Accepted: 8/10/2000 **(NA)**.

Yang, Y., Yan, J., Churchwell, M.I., Beger, R., Chan, P.C., Doerge, D.R., Fu, P.P. and Chou, M.W., Development of a ^{32}P-Postlabeling/HPLC Method for Detection of Dehydroretronecine-Derived DNA Adducts *In Vivo* and *In Vitro*, Chemical Research Toxicology, 14:91-100. Accepted: 11/1/1999 **(E0213301)**.

GENETIC AND REPRODUCTIVE TOXICOLOGY

Director: ***Martha Moore, Ph.D.***
Telephone: **870-543-7050**
Toll Free: **800-638-3321**
E-mail: **mmmoore@nctr.fda.gov**

Sharon Shelton, a research support scientist, measures mutant frequencies in the *lacI* transgene.

Executive Summary

The Division of Genetic and Reproductive Toxicology (DGRT) conducts applied basic research to address specific high priority issues regarding genetic or reproductive toxicology. Division research is directed toward developing and validating new methods that can be used for the identification of potentially hazardous food additives, human and animal drugs, biological therapies and medical devices. In addition, in collaboration with other NCTR scientists, DGRT conducts research to understand the potential toxicity of specific high priority drugs, dietary supplements and/or other agents. Genistein, daidzein, coumestrol and malachite green are currently undergoing extensive evaluations in cross-Division collaborative research efforts.

Currently there are four basic focus areas in the Division research program. Genetic Toxicology research addresses the development of methods to assess the potential for chemicals to negatively impact human genetic material or the function of the genetic material. Reproductive/Developmental Toxicology focuses on methods to understand normal human development and how chemicals might alter normal development. In addition to these disciplinary research areas, the Division conducts research to understand the impact of dietary restriction and dietary supplementation. That research primarily focuses on understanding the physiological and genetic consequences of dietary modulation. Recently the Division initiated a new research focus to utilize new molecular approaches to evaluate genomic damage and alterations in protein production and function. These four focus areas are outlined below.

Genetic Toxicology

Genetic toxicology is the investigation of the ability of chemicals to alter the genetic material. The FDA requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product approval process. Because genetic damage is believed to be important in tumor development, this information is used as a part of the evaluation of suspected carcinogens. Regulatory decisions are based not only on the identification of potentially genotoxic substances, but also on an understanding of their mode of action. Research within the Division centers on the development and validation of new methods by which to assess genetic risk. While tissue culture

approaches are used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity, the Division specializes in the development and validation of *in vivo* mammalian systems. An increased understanding of mutational mechanisms, combined with test systems with an increased ability to detect genetic damage, will provide the FDA with better information for decision-making. As new assays are validated, Division scientists work with international scientists to assure harmonization of protocols and the development of guidelines.

Reproductive/Developmental Toxicology

One of the difficult challenges facing the FDA is the identification and regulation of chemicals, food additives, and biological therapies that may produce birth defects. Such defects affect 7% of the population at birth, another 7% have low birth weights, and at least 25% of pregnancies end in spontaneous abortion. The Division specializes in research to understand how toxicants may induce birth defects such as neural tube defects. Current research addresses the role that the vitamin folic acid may play in the normal closure of the neural tube. This research supports current thinking that diet may play a role in the development of normal offspring and that interactions between diet and toxicants may be important in producing certain birth defects.

A well-defined database created over the past 20 years, led to the initiation of a project to create and validate a computerized knowledge base using quantitative structure activity relationships to predict which chemicals might affect the normal reproductive function.

Diet and Nutrition

Dietary restriction can increase the length of a rodent's life and decrease the frequency of tumors. Division scientists have also determined that decreasing caloric intake alters many physiological processes in rodents and decreases the frequency of mutations. The group has developed many physiological, biochemical, and morphological procedures that can now evaluate dietary modulation. Because of a unique opportunity to collaborate with medical scientists at the University of Tennessee at Memphis, the research program is able to compare the responses seen in rodents with those seen in calorically restricted humans.

Genomics and Proteomics

International research efforts are providing the scientific and medical community with increased understanding of the genome in both humans and rodents. Utilizing this information, new molecular technologies are being rapidly developed and can be used to evaluate structural and functional changes in the genome of both rodents and humans. The Division is developing a new research focus area to use these technologies and to apply them to fundamental risk assessment questions. While current technologies in the field of genetic and reproductive/developmental toxicology generally evaluate single endpoints, these new genomic and proteomic technologies will provide the opportunity to detect alterations in a number of different endpoints.

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Aidoo, Anane

- ***The Frequency and Types of Spontaneous Mutations Found in the Hprt and lacI Genes of Lymphocytes from Transgenic Big Blue Rats*** — ***E0697501*** — ***None*** — ***Predictive Toxicology***

Objective(s):

In vivo assays are used to evaluate whether chemicals have the potential to induce genetic damage (mutations). In order to understand an assay and to use it for hazard identification, one must first understand the frequency and types of mutations that occur spontaneously (without any chemical exposure). This project is designed to evaluate spontaneous mutation in one gene that is in its normal location (*Hprt*) and one gene (*lacI)* that has been genetically engineered into a strain of rats. This strain of rats, containing the *lacI* transgene is called the Transgenic Big Blue Rat. The specific goals of this project are:

1) To determine the frequency of spontaneous mutation at the *hprt* and *lacI* loci in pre-weanling, young (four-month-old) and old (18-month-old) Big Blue rats.
2) To determine the types of mutations present in the mutants.
3) To determine if rats fed different diets have different spontaneous mutant frequencies and if the types of mutations are different.

FY 2000 Accomplishments:

1) Continued sequencing mutants from mice fed different diets – NIH31 and AIN93 *ad libitum* and calorie restricted.
2) Presented poster at the Environmental Mutagen Society meeting and the FDA Science Symposium.
3) Invited presentation at Antimutagenesis Conference, Grand Rapids, Michigan.
4) Two invited seminars at the University of Arkansas at Pine Bluff (UAPB) and the Arkansas Children's Hospital, Little Rock, Arkansas.

FY 2001 Plans:

1) Finish analysis of point mutations in existing mutants.
2) Measure mutant frequencies in CD-1 weanlings and analyze mutations.
3) Prepare manuscripts for publication and submit technical report.

- ***ADDEND: The Frequency and Types of Spontaneous Mutations Found in the Hprt and lacI Genes of Lymphocytes from Transgenic Big Blue Rats*** — ***E0697511*** — ***None*** — ***Predictive Toxicology***

Objective(s):

We have recently developed a method for expanding mutant rat lymphocyte clones from the approximately 100,000 cells per colony that are scored as mutants in our

96-well assay dishes to cultures containing several million cells. It is important to alter the experimental procedure of E0697501 to take advantage of the new technology. Taking advantage of this technology, however, necessitates switching most of the animals used in the project from female to male.

FY 2000 Accomplishments:

This addendum modified E0697501 as to the sex of animals being used in the project. All data reported under E0697501 also applies to this addendum.

FY 2001 Plans:

Same data reported under E0697501 also applies to this addendum.

◆ ***The Use of Antioxidants in Single and in Mixture to Study the Effects of Dietary Vitamins on Genotoxicity Produced in Rats Treated with the Mammary Carcinogen 7,12-dimethylbenz(a)anthracene and the Radiometic Antitumor Drug Bleomycin*** | ***E0701401*** | ***None*** | ***Predictive Toxicology***

Objective(s):

Antioxidants have been reported to have beneficial health effects including reducing the risk of cancer. They have also been reported to reduce the risk of mutation. In this project, dimethylbenz(a)anthracene (DMBA) and bleomycin (BM), both known carcinogens and mutagens, will be administered to rats. Animals will also receive antioxidants (singly and as a mixture of vitamin C, E, β-carotene and selenium). It is expected that the animals treated with both the carcinogen/mutagen and the antioxidants will have a lower mutant frequency than those animals treated only with the carcinogen/mutagen. Two different genotoxic endpoints, mutation at the *Hprt* gene and chromosomal effects (cytokinesis-block micronucleus) will be used.

Once the genotoxicity has been determined, experiments will be undertaken to determine the mechanism underlying the inhibitory action of the dietary antioxidants by determining their effects on:

a) Spectra of induced mutations in *Hprt* gene in lymphocytes.
b) Oncogene (H-*ras*, K-*ras*) and tumor suppressor gene, p53 expression.
c) Programmed cell death (apoptosis).
d) The activities of glutathione peroxidase, and glutathione S-transferase, during DMBA and BM exposures.

FY 2000 Accomplishments:

Experiments are nearly complete and two manuscripts are in preparation. The results of this project were presented at the Antimutagenesis Conference in Grand Rapids, Michigan.

FY 2001 Plans:

A manuscript will be prepared and will be submitted for NCTR internal review.

Title	Project Number	Collaborator	Strategic Research Goal

- ***ADDEND: Evaluation of the Effects of Dietary Antioxidant Intake on Behavior, DNA Damage and Expression of Free Radical Scavenging Enzymes During Physical Exercise in Male and Female Fischer 344 Rats Treated with 2-amino-1-methyl-6-phynelimidazo[4,5-f]pyridine (PhIP)*** — ***E0706311*** — ***None*** — ***Predictive Toxicology***

  Objective(s):

  This addendum will enable the use of both treated and untreated animals to evaluate the effects of dietary antioxidants on animals fed with the known food mutagen PhIP. The additional effect of physical exercise will be evaluated. In addition to the genotoxic endpoints discussed above, this project will also include measurement of mitochondrial DNA mutations. This aspect of the study will make it possible to compare *in vivo* mutations occurring in both nuclear and mitochondrial DNA, as mutations in both systems contribute to human disease burden.

  FY 2000 Accomplishments:

  Protocol reviewed and approved.

  FY 2001 Plans:

  Studies will be initiated to evaluate the effects of physical exercise and dietary antioxidants on mutant frequency.

- ***Evaluation of the Effects of Daidzein and Genistein (Hormone Replacement Agents) on the Genotoxic and Carcinogenic Activity of the Model Mammary Carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) in Ovariectomized Transgenic Big Blue Rats*** — ***E0707001*** — ***None*** — ***Predictive Toxicology***

  Objective(s):

  This project is designed to determine if hormone replacement, in this case the phytoestrogens daidzein and genistein, will alter the risk of mammary tumors. Rats will be exposed to a known mammary carcinogen (DMBA) and given daidzein and/or genistein. In order to model the post-menopausal human situation, the rats will be ovariectomized. In addition to the induction of tumors, other endpoints such as the ability of the DMBA to bind to DNA (DNA adduct analysis) and the frequency and types of mutations induced by DMBA will be investigated.

  FY 2000 Accomplishments:

  This is an externally funded (FDA, Office of Women's Health) project. The Animal Care and Use Committee approved the project in FY 2000 and experimentation should begin in FY 2001.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) Approximately 300 Big Blue rats will be ovariectomized and the treatment will be initiated for this study.
2) Two mutation endpoints, *lacI* mutation in the mammary tissue and *Hprt* mutation in the lymphocytes will be evaluated.
3) Animals that develop tumors (with DMBA) will be screened for mutation in p53 and H-*ras* genes.
4) Modulatory effect of phytoestrogens on mutation frequency spectrum as well as tumor induction and p53 and *ras* mutation frequency will be evaluated.

PI: Chen, Tao

◆ ***Comparison of Mutation Induction and Types of Mutations in the cII Gene of Big Blue Mice Treated with Carcinogens as Neonates and Adults*** — ***X10038*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Because cancer is a disease requiring the induction of mutation and the clonal expansion of mutated cells, one would expect that the developing fetus and young infant would be particularly susceptible to carcinogen exposure. This project will be initiated to evaluate this hypothesis. Experiments will be conducted to:

1) Determine the mutant frequencies in the cII gene of Big Blue mice treated at different ages with direct-acting carcinogens.
2) Determine the mutant frequencies in the cII gene of the target tissues from transgenic mice exposed as neonates and adults to different carcinogens that require metabolic activation.
3) Determine the types of mutations produced in the cII genes of the mutants induced in objectives 1 and 2.

FY 2000 Accomplishments:

This is a new project that will be developed in FY 2001.

FY 2001 Plans:

Develop protocol for evaluating the fetus/newborn/young animal as a sensitive subpopulation for mutagen/carcinogen exposure.

◆ ***Analysis of thymidine kinase (tk) Mutants of L5178Y Mouse Lymphoma Cells*** — ***X10039*** — ***None*** — ***Predictive Toxicology***

Objective(s):

The *in vitro* mouse lymphoma assay is one of the FDA-recommended assays for hazard identification. This assay has been chosen because of the information available supporting its ability to detect a wide variety of genetic alterations. One feature of the assay is induction of mutants that form either small or large colonies in the mutant selection plate. Extensive research indicates that small colony *tk*

mutant formation is associated with chemicals that cause damage to whole chromosomes and large colony *tk* mutant formation is associated with smaller-scale genetic damage. The exact nature of these two types of mutations will be further investigated using the new genomic technology to generate possible hypotheses for further evaluation.

FY 2000 Accomplishments:

This is a new project for FY 2001.

FY 2001 Plans:

This project will be developed and submitted for approval. The specific approach is to:

1) Evaluate large and small colony *tk* mouse lymphoma cell mutants for the specific type of mutational damage.
2) Use microarrays to identify possible genes that may be responsible for the small colony *tk* mouse lymphoma mutant phenotype.

PI: Dobrovolsky, Vasily

◆ ***Validation of the Mouse Targeted $Tk^{+/-}$ In Vivo System for Use in Mutagenicity Studies*** — ***E0701801*** — ***None*** — ***Predictive Toxicology***

Objective(s):

DGRT has developed a new *in vivo* assay for the evaluation of mutant induction. This assay was modeled after the *in vitro* assay, the mouse lymphoma assay, already used internationally for hazard identification. The assay uses the thymidine kinase gene (*tk*) and the *in vitro* assay has been extensively evaluated for its mechanistic basis and shown to detect most, if not all, of the mutational events important to the induction of cancer and other human diseases. This project is designed to further evaluate and validate the *in vivo* assay. Specific goals of this project are:

1) To expand a colony of transgenic $Tk^{+/-}$ mice using breeding of $Tk^{+/-}$ founders and C57Bl/6 mice, and to transfer the $Tk^{+/-}$ genotype to a C57Bl/6 background.
2) To determine spontaneous mutant frequencies at the *Tk* and *Hprt* loci of splenic T-lymphocytes for mice of different ages.
3) To induce mutations in $Tk^{+/-}$ transgenic mice using treatment with the point mutagen ENU (ethyl nitrosourea) and the clastogens BLM and y-radiation, and to measure the kinetics of mutant induction at the *Tk* and *Hprt* loci.
4) To breed transgenic $Tk^{+/-}$ parents in an attempt to derive $Tk^{-/-}$ knockout (KO) mice, and study the biological significance of the *tk* gene in mice.
5) To determine how the $Tk^{-/-}$ genotype may affect mutant frequencies at the *Hprt* locus.

FY 2000 Accomplishments:

1) A manuscript on DMBA-induced mutations in mice was accepted.
2) The colony of mice is being expanded.
3) Experiments were conducted to compare the *Tk* and *Hprt* gene responses.

FY 2001 Plans:

1) In order to expand the use of the assay and to speed its analysis and validation, collaborative studies will be initiated with investigators at other institutions. The NCTR breeding facility will produce and supply mice to others for these studies.
2) Propose to extend the range of mutagens tested in $Tk^{+/-}$ mice through a collaborative arrangement with the Wadsworth Center, NY Dept. of Health, Albany, NY, and the Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.
3) Studies will continue to compare the results obtained using the *Tk* gene with those obtained using the *Hprt* gene.
4) Studies will be conducted to determine mutant frequencies in *Tk*-mice treated by inhalation.

◆ ***ADDEND: Validation of the Mouse Targeted $Tk^{+/-}$ In Vivo System for Use in Mutagenicity Studies*** — ***E0701811*** — ***None*** — ***Predictive Toxicology***

Objective(s):

In order to insure the health of the colony and to produce and supply $Tk^{+/-}$ animals to other investigators, it is necessary to conduct routine microbiological surveillance of the colony to assure that it remains pathogen free.

FY 2000 Accomplishments:

Addendum approved in FY 2000.

FY 2001 Plans:

Surveillance of the colony will be conducted on sentinel animals removed from the colony on an approximately monthly basis, and will consist of tests for the microorganisms listed in the addendum.

◆ ***Evaluation of the $Tk^{-/-}$ Knockout Mouse as a Model of Systemic Lupus Erythematosus*** — ***E0706901*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Our initial experiments suggest that mice deficient in the thymidine kinase enzyme ($Tk^{-/-}$ animals) may be a useful model for studying the human disease lupus. In this project, we will investigate whether the $tk^{-/-}$ genotype in mice is lupus prone. Particular emphasis will be given to documentation of the putative immune-complex mechanism of the renal disease, and in-depth evaluation of the immune system in $Tk^{-/-}$ mice, seeking comparison with published characteristics of Systemic Lupus Erythematosus in mice and humans.

FY 2000 Accomplishments:

This protocol was just recently approved. We have the mouse colony ready for expansion.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

To produce mice of desired genotype and begin testing them for the presence of antibodies.

◆ ***A Novel Transgenic Model for Mutation Detection Using Fluorescent Markers*** — ***X90027*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Current methods to select for rare mutations in a population of normal cells require extensive culturing of cells and the use of drugs that allow the mutant, but not the normal cells, to grow. These methods are subject to technical difficulties and are time-consuming and expensive. This project is directed toward developing a new approach to mutation detection using fluorescent markers.

FY 2000 Accomplishments:

Three CHO cell lines were engineered that contain transgenes needed for novel systems for mutation detection. The cell lines are under evaluation at this time.

FY 2001 Plans:

To finish the evaluation of two transgenes system *in vitro*. If results are promising, we may consider an *in vivo* analog.

PI: Domon, Olen

◆ ***Evaluation of the Genotoxicity of the Phytoestrogen, Coumestrol, in Human Lymphoblast Cells that Differ in the Mutational Status of the p53 Tumor Suppressor Gene*** — ***E0705501*** — ***None*** — ***Predictive Toxicology***

Objective(s):

The phytoestrogens are considered to be potentially beneficial dietary supplements, particularly for peri- and post-menopausal women. The phytoestrogen coumestrol will be evaluated in several *in vitro* gene mutation assays with the following goals:

1) To confirm the ability of coumestrol to break chromosomes. This will be done using the micronucleus assay.
2) To confirm the mutagenicity of coumestrol at the *HPRT* locus.
3) To determine if coumestrol induces large-scale chromosomal damage such as that detected by the *Tk* mutation assay.
4) To determine if the toxicity of coumestrol is due to apoptosis.
5) To determine the effect of coumestrol on the ability of cells to grow and divide normally.

FY 2000 Accomplishments:

Performed mutation assay, micronuclei assay, measured apoptotic cells by viability assay, identified mutants, and expanded both normal and slow growth for doing molecular biology.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Continue molecular analysis of mutants.

◆ ***ADDEND: Evaluation of the Gentoxicity of the Phytoestrogen, Coumesterol, in Human Lymphoblast Cells that Differ in the Mutational Status of the p53 Tumor Suppressor gene. I. Molecular Analysis of Coumestrol and Genistein-induced Thymidine Kinase Mutants in AHH-1 $Tk^{+/-}$ and L3 Cells***

E0705511 | *None* | *Predictive Toxicology*

Objective(s):

In the master project, cells in culture were treated with coumestrol and genistein, and clones of cells that were made mutant at the thymidine kinase gene were isolated. In this addendum, these mutants will be evaluated to determine the specific types of mutations that were induced by these two phytoestrogens.

FY 2000 Accomplishments:

Presented poster at the Environmental Mutagen Society meeting in New Orleans describing the mutants induced by coumestrol and genistein.

FY 2001 Plans:

1) Continue molecular analysis of mutants.
2) Confirm the sequence of the Thymidine kinase gene in AHH-1 $Tk^{+/-}$ and also in the L3 cell line.
3) Determine the nature of the mutation in genistein-induced and coumestrol-induced *Tk* mutant clones.

PI: Duffy, Peter

◆ ***Effect of Different Levels of Caloric Restriction (CR) on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition Variables in Rats***

E0692401 | *CFSAN* | *Concept-Driven*

Objective(s):

1) To determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc.
2) To determine the relationship between body fat (BF), fat free mass (FFM), total body water (TBW), and total body electrical conductivity (TOBEC) as a function of strain, age, mass, and nutritional status in rats.
3) To validate and automate the use of a new, non-invasive electromagnetic scanning device to measure BF, FFM, and TBW and to compare the results to a conventional chemical fat extraction technique.
4) To determine if CR alters the relative quantity and disposition of various types of

lipids such as cholesterol, phospholipids, free fatty acid, etc., in various tissues, as well as in urine, feces, and blood serum.

5) To develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low calorie foods, food additives, and food substitutes.
6) To determine temporal and environmental factors that modulate the effects of CR.
7) To develop experimental methods for utilizing a low level of CR to increase the survival rate and to decrease variablity in the chronic bioassay; to provide the concomitant control data for comparison.
8) To develop control data for a reference purified diet that has been formulated to conform to the long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.

FY 2000 Accomplishments:

The main objectives were to determine if low levels of dietary restricted (DR)-induced stress could be used to increase the survival rate of SD rats in the chronic bioassay and to identify the survival characteristics of a non-obese SD rat strain (NCTR colony).

1) A study was undertaken to determine the effects of stress resulting from incremental levels of dietary restriction (DR) in rats. Survival, growth, reproductive, physiological, behavioral and dietary-intake variables were monitored in a chronic study in which male Sprague-Dawley (SD) rats (NCTR colony) were fed the NIH-31 cereal diet at three levels of DR.
2) In a second study, survival, growth, physiological, behavioral, and dietary-intake variables were monitored in a chronic two-year study in which male Sprague-Dawley (SD) rats (NCTR colony) were fed different amounts of the AIN-93M purified diet. The main objectives were to ascertain the suitability of the AIN-93M diet for use in chronic bioassays and to determine if DR significantly increases the longevity of rats fed this diet containing the protein, casein. The data acquisition phase of these nutritional studies was nearly completed in the SD rat. Only the two-year pathology study is yet to be completed in FY 2001.

FY 2001 Plans:

During FY 2001, the research protocols (see FY 2000 Accomplishments above) will be replicated using the Fischer 344 rat. This strain is very important to the FDA because it is routinely used in NTP studies. The USDA and CFSAN plan to use the results of this study to develop future studies to determine the effects of food additives and supplements.

◆ ***ADDEND: Task Order #483 & #493 - LIMS Implementation and Review of Heart Rate Variation Analysis Software*** — ***E0699811*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Addendum requested to add ADP resources needed for Task Order #483 - Memphis Study: LIMS Implementation

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

A database to support the Memphis study on human obesity was initiated and the development stage was nearly completed in the past year. A battery of physiological parameters such as energy utilization, respiratory quotient, body temperature, heart rate, and activity and behavioral variables such as memory, visual and auditory acuity, motor reflex, and pulmonary function were performed on 28 obese and dietary-restricted patients. The data collection phase of the study will be completed in the next three months. The preliminary results suggest that the beneficial physiological effects of dietary restriction in humans such as lower body temperature and heart rate, and increased metabolic efficiency are similar to those previously reported for rodents. These results may suggest that dietary restriction may increase longevity and decrease age-related diseases and drug toxicity in humans.

FY 2001 Plans:

The data collection phase of the study will be completed for 43 patients and data-analysis phase of the project will be finalized in FY 2001. Manuscripts will be prepared and submitted for publication.

◆ ***Study to Develop and Evaluate Appropriate Diets and Animal Models for Chronic Bioassay Studies*** — ***X10041*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To evaluate and compare growth, survival, physiological, and pathological profiles in rodents that are fed a maintenance-formulated purified diet or the Harland 14% protein plant-based diet.
2) To selectively modify the vitamin component concentrations of the AIN-93M purified diet to increase rodent survival rate in the chronic bioassay to conform to the FDA's "Redbook" guideline.
3) To determine if male and female rats fed these diets have similar life spans and pathology.
4) To determine if genetic and molecular variables such as mutant frequency, free radical production and oxidative damage, DNA repair, expression of genes encoding drug metabolizing enzymes and other biomarkers of toxicity and proto-oncogene expressions are sensitive and accurate biomarkers that can predict the survival potential of rats in chronic bioassay studies.
5) To determine how dietary restriction interacts with various nutritional components to alter aging and disease processes.

FY 2000 Accomplishments:

The experimental protocol for this study is being prepared. No experimental work was done in FY 2000.

FY 2001 Plans:

The final protocol will be completed and the preliminary phase of the new nutritional study will be initiated in FY 2001. We plan to develop a CRADA with CFSAN,

USDA, and industry to partially fund this protocol. The primary objective of this chronic study is:

1) To evaluate growth performance, survival, and physiological, biochemical, genetic, and pathological profiles in rodents that are associated with a new purified diet (AIN-93M) and the Harlan vegetarian diet to determine if these diets are appropriate for use in chronic bioassay studies. Purified diets are optimal for nutritional studies because they can be formulated to precise specifications.
2) To selectively modify the nutritional components of the AIN-93M purified diet to increase the survival rate of rodents in the chronic bioassay to conform to the FDA's "Redbook" guideline.
3) To identify long-lived strains of rodents for chronic bioassay studies.
4) To determine if the male and female rats raised on the various diets have similar life spans and disease profiles.
5) To determine appropriate levels of dietary restriction (DR) to increase the survival rate of rats in the chronic bioassay without changing the baseline sensitivity to carcinogenesis.
6) To ascertain basic mechanisms by which DR interacts with various nutritional components to modulate aging, disease, and drug toxicity. We will determine if the SIR2 gene, a gene that has been shown to interact with DR to increase longevity in yeast, can increase the life span of rats by protecting their DNA from the effects of oxidative damage.

One of our working hypotheses to be tested in this study is that the addition of vitamin supplements to the purified AIN-93M diet will increase the survival potential of rats in chronic studies to conform to FDA regulations for studies that require a synthetic diet.

PI: Feuers, Ritchie

◆ ***Influence of Dietary Restriction on Somatic Mutation and Antioxidant Enzymes Induced by Exposure of Female and Male Fischer 344 Rats to Bleomycin (BLM)*** — ***E0699101*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Dietary restriction has been shown to decrease the frequency of spontaneous mutants. In this project rats will be exposed to a known mutagen (bleomycin) and evaluated for the effect of dietary restriction on the frequency of induced mutation and on the antioxidant enzymes. Specifically experiments will be conducted:

1) To determine the frequency of occurrence of lymphocytes bearing a mutant form of the *Hprt* gene as an indicator of DNA damage in caloric restricted and in *ad libitum* rats following exposure to bleomycin (BLM).
2) To determine how the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione reductase relates to the mutant frequencies determined from the above objective.

3) To determine the activity of the electron transport systems as an indicator of mitochondrial function during drug exposure.
4) To evaluate the integrity of mitochondrial DNA in BLM-treated rodents.

FY 2000 Accomplishments:

1) All phases of animal treatment were successfully completed, and sample and data analyses are complete.
2) Two manuscripts have been published.

FY 2001 Plans:

Another manuscript will be completed, a technical report prepared and the study closed.

◆ ***Memphis Study: Evaluation of Calorically Restricted Human Surgical Samples Received from Department of Surgery University of Tennessee, Memphis*** — ***E0699801*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.

FY 2000 Accomplishments:

1) The goal of the study is to determine if physiologic, metabolic, biochemical and molecular changes that occur with caloric restriction (CR) in rodents change in similar ways in humans who have undergone a surgical procedure that produces CR.
2) During FY 2000 an additional 24 patients (43 total) were added to the study. Thus, the study should be nearing completion by December 2000. If this goal is met, only one year of two-year follow-up will be needed.
3) Nutritional evaluations (through 24 human recall interviews) were completed for all patients prior to surgery, 3, 6, and 12 months post surgery. This provides precise and critical data on caloric consumption, and nutrient content of each patient's diet.
4) Detailed procedures have been optimized for physiological evaluation of each patient, and a comprehensive computer networking system has been put in place which allows for interaction among clinical, physiological, metabolic, biochemical and molecular data bases.
5) During FY 2000 detailed analysis of oxidative phosphorylation in mitochondria from lymphocytes has been completed.

FY 2001 Plans:

The last patients will be sampled and physiologic evaluations made by the end of the year. The remainder of FY 2001 will be used to finish biochemical analysis, data analysis, and manuscript preparation. It is currently planned to finish this study by the end of FY 2001.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Methods Development in Proteomics***	***X10033***	***None***	***Predictive Toxicology***

Objective(s):

To establish the capability to utilize the new proteomic technology for toxicological evaluations.

FY 2000 Accomplishments:

Literature reviews and mapping strategy for methods development preliminary study are being completed.

FY 2001 Plans:

Prepare and initiate a P-number preliminary project to develop the basic technology to put in place state-of-the-art methods for proteomics as a research tool at NCTR.

PI: Hansen, Deborah

◆ ***Antisense Knockouts of Genes in the Folate Pathway and Effects on Neural Tube Development***	***E0702001***	***None***	***Predictive Toxicology***

Objective(s):

The Division specializes in research to understand how toxicants may induce birth defects such as neural tube defects. This research project addresses the role that the vitamin folic acid may play in the normal closure of the neural tube. This research supports current thinking that diet may play a role in the development of normal offspring and that interactions between diet and toxicants may be important in producing certain birth defects. The research addresses fundamental mechanisms by which the enzymes that metabolize folic acid may be involved and whether the addition of folic acid or other vitamins would overcome the toxicity of and result in normal neural tube closure and thus in normal offspring. The specific goals of the project are:

1) To determine if knocking out 5,10-methyltetrahydrofolate (MTHFR) enzyme activity in mouse embryos *in vitro* produces neural tube defects.
2) To determine if addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MTHFR activity and produce normal closed neural tubes in mouse embryos treated *in vitro*.
3) To determine if the addition of methionine overcomes the lack of MTHFR activity and results in normal closed neural tubes in mouse embryos treated *in vitro*.
4) To determine if knocking out methionine synthase (MS) activity in mouse embryos *in vitro* produces neural tube defects.
5) To determine if the addition of methionine is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated *in vitro*.
6) To determine if exogenous vitamin B12 is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated *in vitro*.

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

7) To determine if knocking out methionine adenosyltransferase (MAT) enzyme activity in mouse embryos *in vitro* produces neural tube defects.
8) To determine if addition of exogenous methionine is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*.
9) To determine if addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*.

FY 2000 Accomplishments:

Thus far, we have modulated expression of 5,10-methylenetetrahydrofolate reductase, folate binding protein-1 and a regulatory factor necessary for expression folate binding protein-1. Decreased expression of each produced neural tube defects. We have some preliminary information on decreased expression of cystathione B-synthase and folate binding protein-2. We also have used the micro-injection technique to determine that homocysteine, when injected into the amniotic sac, did not adversely affect neural tube development. Three manuscripts have been derived from these data, one of which has been accepted for publication and the other two submitted to journals for consideration.

FY 2001 Plans:

Over the next year, we plan to complete the antisense work on cystathionine-synthase, folate binding protein-2 and to decrease expression of methionine synthase and methionine adenosyltransferase. In addition to the embryo culture work, we need to do reverse transcriptase–polymerase chain reaction (RT-PCR) in order to insure that expression has been decreased, to look at protein levels with antibodies (if available), and to do *in situ* hybridization to verify embryonic expression of the genes.

◆ ***Indices of Biotin Nutrition*** ***E0703401*** ***None*** ***Concept-Driven***

Objective(s):

To determine the human requirement for biotin in normal individuals and in individuals in certain circumstances in which biotin status may be impaired. We will determine whether biotin of similar severity to that observed in human pregnancy can cause significantly increased rates of fetal malformation in the mouse. In the pilot mouse study, marginal biotin deficiency in mouse dams that caused an increase in 3-HIA excretion similar to that seen in human pregnancy produced 10% incidence of cleft palate in the fetal mouse.

FY 2000 Accomplishments:

Data collected during FY 1999 continue to be analyzed by our collaborators at the UAMS. An abstract was submitted late in 1999, but no other work has been published during FY 2000.

FY 2001 Plans:

It is anticipated that very little work on this protocol will be forthcoming from NCTR during FY 2001.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Predictability of Animal Data for Human Developmental Toxicity***	***E0703501***	***CDER***	***Predictive Toxicology***

Objective(s):

To determine if the animal tests currently required for pre-market approval of drugs are adequately predicting possible developmental toxicity risk for humans. This is a collaborative project with CDER in which the already-existing data are abstracted and utilized to help determine whether animal data can be used to predict human health. Both the published literature and data in CDER files will be utilized. The specific strategy for conducting this analysis is as follows:

1) To retrieve reports of human data from published literature and FDA files for therapeutic agents for which there are adequate data to indicate either positive effects or no effect.
2) To retrieve reproductive and developmental toxicity study data in laboratory animals from FDA files or directly from pharmaceutical companies on the same products.
3) To extract specific data elements into a database for qualitative and quantitative comparison.
4) To evaluate data using the expertise of pharmacology/toxicology and clinical/epidemiology project participants.
5) To conduct statistical analyses, initially using multiple-regression analyses and correlation approaches, with more sophisticated analyses as the data permit.
6) To draw conclusions about the predictability of animal testing data and recommend design improvements as appropriate.

FY 2000 Accomplishments:

1) We are currently collecting data from the FDA files on various drugs that are used during human pregnancy and that may or may not cause developmental toxicity in humans.
2) We are also collecting data from the literature as well as from FDA adverse effects reporting systems on any adverse effects of the drugs if used during human pregnancy. During FY 2000, data were collected on valproic acid, enalapril, and fluoxetine.
3) Three preliminary reports (abstracts) of some of the data were presented at the annual meeting of the Teratology Society.

FY 2001 Plans:

We plan to continue to collect data from both the literature as well as FDA files on additional compounds. These data will be analyzed statistically to determine if the animal tests as currently required adequately predict risk for adverse developmental outcomes in humans.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Mechanism(s) of Folate-Responsive Dysgenesis***	***E0707401***	***None***	***Concept-Driven***

Objective(s):

In this project the specific mechanisms by which folate impacts the events that lead to the closure of the neural tube during fetal development will be investigated. The specific aspects and goals of the project are:

1) To determine if there is concordance between the expression of the folate receptor (FBPI) and the most proliferative cohorts of neural tube- and neural crest-cells during defined 12-hour windows on each day of gestation from GD 5 to GD 15, and to determine if the loss of these cohorts of cells during these windows of antifolate exposure gives rise to recognizable neural tube defects and neurocristopathies in the fetus at term.
2) To characterize the basal expression of FBPI isoforms; and extent and mechansim of FBPI regulation in the placenta and various fetal tissues on GD 17 among cohorts of dams fed a folate-deficient or folate-replete diet.
3) To determine if sustained quenching of placental cytotrophoblast FBPI by antisense FBPI cDNA overexpression from GD 8 to GD 16 during maternal folate deficiency has an adverse impact on cytotrophoblastic proliferation leading to small placentas and global growth retardation of fetuses.
4) To demonstrate that neural tube closure and neural crest cell function in the whole mouse embyro at GD 8.5 can be perturbed by down-regulating FBPI expression in neural tube cells through the introduction of antisense oligonucleotides to the 43-kDa trans-factor which is required for FBPI transcription.

FY 2000 Accomplishments:

1) Using animals on E0702001, we used antisense sequences to decrease expression of the folate receptor (folate binding protein-1 in the mouse).
2) These data were used as preliminary evidence for a hypothesis that was developed jointly by Indiana University and the NCTR.
3) This hypothesis was published.
4) These preliminary data were also used as support for the grant proposal that was submitted.

FY 2001 Plans:

This collaborative project was recently funded by NIH (award letter received 09/01/00; funding for 5 years) and has been submitted to NCTR as a protocol that is currently being reviewed for animal care and use approval. Experiments should be initiated in FY 2001.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Harris, Angela

- ***Modulation of Gene Expression in Chemical Carcinogenesis: Analysis of Aflatoxin B_1 Induced Gene Expression in Human Hepatocytes*** — *E0704701* — *None* — *Concept-Driven*

Objective(s):

This project is a part of the DGRT genomic/proteomics focus area. The goal of this project is to utilize the new gene expression technologies to understand which genes are affected by the exposure of human hepatocytes to a known human carcinogen (aflatoxin E_1). Because this is a new technology, the experimental approach must include research to define the appropriate experimental parameters. Specific goals are to:

1) Verify aflatoxin B_1 effects on steady-state mRNA levels of eight genes previously identified by differential hybridization of a gene filter array to be aflatoxin B_1 (AFB_1)-responsive in human hepatocytes.
2) Use Northern blot, RT-PCR, and/or RNA protection assay to establish AFB_1 time- and dose-response curves for maximal gene expression and also determine the minimum dose at which gene expression can be detected.
3) To identify additional AFB_1-induced genes using differential display PCR (DD-PCR) and differential hybridization of a high-density filter array utilizing mRNA from human hepatocytes treated with low, moderate and cytotoxic levels of AFB_1.
4) To evaluate selected genes as described for Objective 1.
5) To distinguish genes involved in toxicological response to AFB_1 exposure from those that contribute to the carcinogenic response by comparing the gene expression profile of human hepatocytes treated with the hepatotoxic non-carcinogenic chemical, acetaminophen.
6) To compare gene expression of selected genes in human hepatocytes treated with known rat liver chemical carcinogens, including 2-acetylaminofluorene, dimethylnitrosamine and methapyrilene.

FY 2000 Accomplishments:

1) Human hepatocytes from four donors were treated with aflatoxin E_1 (AFB_1) or acetaminophen (APAP). Total RNA, DNA and protein were isolated from all samples. The RNA was used to analyze gene expression on Clontech Tox/Stress Filters (274 genes/filter) and/or Genome Systems GDA filters (18,000 genes/filter).
2) Gene expression data were analyzed using Array Vision.
3) Hep2 cells were treated with AFB_1. Total RNA, DNA and protein were isolated from all samples. Total RNA was used to analyze gene expression on Genome Systems GDA filters. The data are currently being analyzed to determine the effects of AFB_1 and to compare and contrast with data from normal primary hepatocytes. Northern blot analysis of selected clones in HepG2 RNA is ongoing.

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

4) Hepatocytes from six rats were isolated and plated on different matrices to determine the effects of various plating conditions on gene expression. In addition, the hepatocytes were treated with methapyrilene and pyrilamine to determine effects of these two antihistamines on gene expression. Gene expression was analyzed using Research Genetics rat filters (5000 genes/filter). Northern blot analysis of 17 genes in 13 data sets is 75% complete.
5) Presented the data from #3 as a talk at the Seager-Braswell Graduate Student Research Symposium at the UAMS in March 2000. It was also presented as a poster at the Environmental Mutagen Society meeting in New Orleans.

FY 2001 Plans:

1) Write and submit the three manuscripts that are the result of accomplishments #1-3 above.
2) Procure human hepatocyte donors from three females to analyze AFB_1-responsive genes and compare to the data from the male donors.
3) Procure human hepatocytes from three additional male donors to analyze the effects of 2-acetylaminofluorene (2-AAF) and dimethylnitrosamine (DMN). Compare to AFB_1 and APAP data.
4) HepG2 cells will be treated as in #3 for comparison.
5) Present AFB_1 human data at the Society of Toxicology meetings in San Francisco, California and at the UAMS.

◆ ***ADDEND: Task Order #721: MicroArray Data Management System (ArrayTrack)*** ***E0704711*** ***None*** ***Concept-Driven***

Objective(s):

To develop software, ArrayTrack, for microarray data management and knowledge mining. The gene expression data technology results in extremely large amounts of data that must be analyzed. This project will develop software that can assist the capture and analysis of data.

FY 2000 Accomplishments:

Developed a web-based array database to store image files, raw data, processed data, and other relevant information that can be accessed by any interested/authorized party.

FY 2001 Plans:

1) Design and construct a database system to store heterogeneous microarray data.
2) Provide user-friendly input window for experiment data.
3) Transform the array information (the cDNA clones of each spot on the array) from manufacture-web-site to database, including Uni-Gene, Gene Bank, and dbEST entry numbers when available.
4) Embed browser buttons within the spreadsheet to load/save files, including image, template, and intensity text files. Import and export for intensity data as a group are optional at request.
5) Integration and enhancement of user-interface.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Hass, Bruce

- ***Development of a Keratinocyte HPRT Mutation System*** — ***P00421*** — ***None*** — ***Other Research***

Objective(s):

The exposure of individuals to ultraviolet (UV) light or the photoactivation of chemicals to UV light is an important human health hazard. NCTR has a state-of-the-art facility to expose animals or cells in culture to UV light and to evaluate the possible adverse outcomes. UV light is known to cause mutations and also to cause skin cancer in humans. This project will investigate the possible use of a human keratinocyte (a type of skin cell) cell line for the detection of mutation. The *HPRT* gene will be used as the target marker gene.

FY 2000 Accomplishments:

Protocol approved. The human keratinocyte cell line (HaCaT) obtained. Cell parameters are being determined: cloning efficiency and cellullar growth rate. In addition the cells are being evaluated for DMSO and acetone toxicities. These two chemicals are common solvents used for applying test substances to cells in culture.

FY 2001 Plans:

1) Validate human keratinocyte *HPRT* assay using known mutagens.
2) Evaluate UV and UV in combination with chemicals.

PI: Heflich, Robert

- ***Effect of Maternal Diet in DNA Damage, Oxidative DNA Adducts and Lymphocyte HPRT Mutation in Human Cord Blood*** — ***X10031*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Human lymphocytes can be obtained from the umbilical cord during the birth of an infant. It is then possible to evaluate these lymphocytes for the presence of genetic damage. The Division has several projects evaluating the impact of diet on the induction of mutation. This project will provide an opportunity to evaluate the impact of diet on the induction of mutations in humans.

FY 2000 Accomplishments:

1) Initiated planning experiments.
2) Obtained agreement for collaborations with scientists in Pune, India; NCTR; and Arkansas Children's Hospital in Little Rock, Arkansas.

FY 2001 Plans:

Prepare and submit NCTR protocol for approval.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Effect of Azathioprine in Somatic Cell and Germline Hprt Mutant Frequencies in the Mouse***	***X10057***	***None***	***Predictive Toxicology***

Objective(s):

Test the hypothesis that *in vivo* selection by azathioprine affects both somatic cell and germline *Hprt* mutant frequencies using the mouse. This project is based on the theoretical possibility that the drug azathioprine would increase the number of *HPRT* mutants in the cells of humans being treated with the drug. This increase in mutant frequency would be predicted to, ultimately, in future generations, result in an increase in Lesch-Nyhan-affected individuals. The *Hprt* mutation mouse model provides an opportunity to test this hypothesis.

FY 2000 Accomplishments:

This is a new project under development. No work was completed in FY 2000.

FY 2001 Plans:

Prepare and submit protocol for approval.

PI: Khaidakov, Magomed

◆ ***The Development of a Genotypic Selection Assay and Analysis of the Age-Specific Patterns of Mutant Accumulation***	***E0706301***	***None***	***Predictive Toxicology***

Objective(s):

The majority of methods for quantitating mutation require the use of selective drugs that allow mutants to grow and prevent normal cells from growing. All of these techniques require extensive cell culture and can be time-consuming and expensive. There are techniques that utilize genotypic selection that allow for a molecular amplification of the rare mutant DNA sequences and thus provide for a direct measurement of mutant frequencies. The Division has several projects in which this new technology is being developed. The specific goals of this project are:

1) To develop a genotypic selection assay (GSA) allowing a direct measurement of mutant frequencies and molecular analysis of mutation in any non-polymorphic endogenous sequence and in any tissue.
2) To determine the spontaneous mutant frequencies (MFs) and age-associated accumulation rates (ARs) in highly (Exon 3) and poorly (Exon 4) mutable regions of *hprt* coding sequence in the *hprt* lymphocyte mutation assay.
3) To compare the *in vivo* persistence of elevated Mfs in *hprt* exons 3 and 4 induced after exposure to ethyl nitrosourea (ENU).

FY 2000 Accomplishments:

1) An assay for analysis of mutations in mitochondrial DNA is established.
2) Several samples from experiments E07014 and E06924 were analyzed and the mutations evaluated by sequencing.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) To analyze human and animal samples using this assay.
2) Age-related changes, caloric restriction will be evaluated.
3) Mutagen-induced changes will be evaluated.

◆ ***ADDEND: Evaluation of the Effects of Dietary Antioxidant Intake on Behavior, DNA Damage and Expression of Free Radical Scavenging Enzymes During Physical Exercise in Male and Female Fischer 344 Rats Treated with 2-amino-1-methyl-6-phynelimidazo[4,5-f]pyridine (PhIP)*** — ***E0706311*** — ***None*** — ***Predictive Toxicology***

Objective(s):

This addendum will enable the use of both treated and untreated animals. We also intend to include measurement of mitochondrial DNA mutations as an additional end-point to the nuclear DNA mutations already described in the main protocol. This aspect of the study will make it possible to compare *in vivo* mutations occurring in both nuclear and mitochondrial DNA, as mutations in both systems contribute to human disease burden.

FY 2000 Accomplishments:

Protocol prepared and submitted for review.

FY 2001 Plans:

Experiments will begin once the protocol has been approved.

PI: Laborde, James

◆ ***Dose-Response of Retinoic Acid (RA)-Induced Stress Protein (SP) Synthesis and its Correlation with Developmental Toxicity in CD-1 Mice*** — ***E0697601*** — ***None*** — ***Concept-Driven***

Objective(s):

1) Determine the incidence of limb malformations on gestation day 17 (GD 17) and the extent of synthesis of SPs in limb bud tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD 11.
2) Determine the incidence of cleft palate on gestation day 17 (GD 17) and the extent of synthesis of SPs in craniofacial tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD 13.

FY 2000 Accomplishments:

The work from this project has resulted in one manuscript that was submitted.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Project is complete, no additional work will be done.

◆ ***ADDEND: Dose-Response of Retinoic Acid-Induced Stress Protein Synthesis and Its Correlation with Developmental Toxicity in CD-1 Mice: T.O. #490 (Ident. and Analysis of GEL Image Blots)*** — *E0697611* — *None* — *Concept-Driven*

Objective(s):

Addendum submitted to cover ADP Task Order #490 requesting ADP resources.

FY 2000 Accomplishments:

Manuscript accepted and will be published in January 2001.

FY 2001 Plans:

Project has been completed and the technical report will be submitted.

PI: Lu, MingHsiung

◆ ***ADDEND: Evaluation of Cellular Responses in Rats -- Cell Proliferation Study by Flow Cytometric Cell Cycle Analysis*** — *E0692421* — *CFSAN* — *Concept-Driven*

Objective(s):

Dietary restriction may affect the growth of cells in various organs. This project will use flow cytometric cell cycle analysis for cell proliferation studies to determine if cell growth is impacted by dietary restriction Samples from earlier studies will be used. Bone marrow, kidney, spleen, and thymus tissues will be analyzed to determine any cell proliferation activities of tissues obtained from rats that received various levels of dietary restriction.

FY 2000 Accomplishments:

Investigated the effect of different levels of dietary restriction on cellular proliferation in bone marrow.

FY 2001 Plans:

1) Other tissues of interest such as esophageal epithelial tissue will be assayed.
2) Work on data calculation, statistical analyses and comparisons will be performed.

PI: Manjanatha, Mugimane

◆ ***ADDEND: Micronucleus and Gene Mutation Analysis in F344 Big Blue Rats Administered Leucomalachite Green in the Diet for 4, 16, and 32 weeks*** — *E0212821* — *None* — *Agent-Driven*

Title	Project Number	Collaborator	Strategic Research Goal

Objective(s):

Malachite and leucomalachite green are currently being tested for carcinogenicity under the National Institute of Environmental Health Sciences Interagency Agreement (IAG). The objective of this project is to assess the mutagenicity of leucomalachite green in relation to DNA adduct formation in tissues of Big Blue rats.

FY 2000 Accomplishments:

This proposal was approved to begin in FY 2001.

FY 2001 Plans:

Evaluate leucomalachite green genotoxicity in Big Blue rats: Four doses and three time points will be used and the *lacI* mutant frequency in the liver tissue will be determined along with the *Hprt* mutant frequency in the lymphocyte and micronucleus assay in the bone marrow.

◆ ***Quantitative and Molecular Analysis of 7,12-dimethylbenz[a]anthracene-induced Mutations in the Model Blue Rat: Comparison of Mutagenesis in the Transgene lacI with the Endogenous Gene Hprt and Cancer Genes H-ras and p53*** — ***E0690601*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To determine the mutant frequency and mutation spectrum of the *lacI* transgene of the Blue Rat following exposure to DMBA in surrogate and target tissues and compare these mutant frequencies and mutational spectra to those determined in Objectives 2 and 3.
2) To determine the mutant frequency and mutation spectrum of the endogenous *Hprt* reporter gene in T-lymphocytes from the spleens of Fischer 344 and Blue Rats following exposure to DMBA.
3) To induce mammary tumors in Fischer 344 rats and Blue Rats by exposure to DMBA and screen tumor DNA for mutations in the oncogene, H-*ras* and the tumor suppressor gene, p53.

FY 2000 Accomplishments:

1) This was a long-term project concluded in July 2000.
2) The technical report was prepared.
3) Several manuscripts generated by this project have been published, two of which were published in FY 2000.

FY 2001 Plans:

This project will not be continued beyond FY 2000. A final manuscript derived from this project is under preparation. The project will be terminated.

Title	Project Number	Collaborator	Strategic Research Goal

- ***ADDEND: Quantitative and Molecular Analysis of 7,12-dimethylbenz[a]anthracene-induced Mutations in the Model Blue Rat: Comparison of Mutagenesis in the Transgene lacl with the Endogenous Gene Hprt and Cancer Gene H-ras*** — ***E0690611*** — ***None*** — ***Predictive Toxicology***

  Objective(s):

  NCTR protocol E0690601 was undertaken in order to validate the use of the Big Blue rat as a model for determining the *in vivo* mutagenicity of potential human toxicants.

  FY 2000 Accomplishments:

  Results from experiment 1 uncovered unanticipated issues concerning the nature of mutagenic responses in the Big Blue model. These results suggest experiments not included in the original protocol that may resolve these issues. Necessitates using additional animals to complete experiments 1, 2, and 3.

  FY 2001 Plans:

  The technical report will be written when the addendum is completed.

- ***A Study of DNA Repair in the Transgene of Big Blue Rats*** — ***X70026*** — ***None*** — ***Predictive Toxicology***

  Objective(s):

  The *lacI* background mutant frequency varies widely among tissues. It is particularly puzzling why it should be very low in stem cells such as bone marrow. The Division will to investigate possible reasons for these differences. Possible factors may include: 1) methylation status, and 2) transcription of the *lacI* gene and transcription coupled repair (TCR). Scientists are developing the reverse transcriptase–polymerase chain reaction (RT-PCR) evaluation that will allow one to look for the expression of *lacI* message in different tissues.

  FY 2000 Accomplishments:

  This is a new project under development.

  FY 2001 Plans:

  A protocol will be developed and submitted for approval.

PI: Mckinzie, Page

- ***Application of the MutEx/ACB-PCR Method of Genotypic Selection to the Detection of K-ras Mutations*** — ***E0706601*** — ***None*** — ***Predictive Toxicology***

  Objective(s):

  The majority of methods for quantitating mutation require the use of selective drugs which allow mutants to grow and prevent normal cells from growing. All of these techniques require extensive cell culture and can be time-consuming and expensive.

There are techniques that utilize genotypic selection that allow for a molecular amplification of the rare mutant DNA sequences and thus provide for a direct measurement of mutant frequencies. The Division has several projects in which this new technology is being developed. The specific goals of this project are to:

1) Establish assays that can provide mechanistic data for chemical risk assessment and aid in establishing the relevance of rodent models for predicting human risk. The proposed research approach is to apply a recently developed method, MutEx/ACB-PCR to the detection of human and rodent k-*ras* GGT->GAT and GGT->GTT mutations. The assays will then be used to study the chemical induction of these mutations.

FY 2000 Accomplishments:

1) Cloning of human k-*ras* wild type.
2) Mutagenesis of human k-*ras* from GGT to GAT and GGT to GTT.
3) Cloning of rat wild-type k-*ras*.

FY 2001 Plans:

1) Mutagenesis of rat k-*ras* to make GGT to GAT and GGT to GTT mutants.
2) Finish optimization of human and rat ACB-PCR.
3) Optimize MutEx method for human and rat k-*ras* studies.
4) Begin validation of method with unknown samples.

PI: Mittelstaedt, Roberta

◆ ***ADDEND: Measurement of H-ras Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection*** — ***E0704121*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Quantify and identify *lacI* mutations in liver DNA of mice treated as neonates with 4-aminobiphenyl in order to establish mutation induction and specificity as an early event in hepatic tumorigenesis.

FY 2000 Accomplishments:

1) An addendum to E0704101 was written and approved. This addendum encompasses the characterization of mutations induced in the *lacI* gene of Big Blue mice treated with 4-aminobiphenyl.
2) The treatment of Big Blue mice as newborns has been completed.
3) The collection of tissues from the 4-aminobiphenyl-treated and control Big Blue mice associated with this protocol is nearly complete.

FY 2001 Plans:

1) Complete the tissue collection from the treated animals.
2) Perform the *lacI* mutational analysis and publish the results.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Morris, Suzanne

- ***p53 Transgenic Mouse Evaluations of Genistein: 28-day and 36-week Studies*** — ***E0213601*** — ***None*** — ***Predictive Toxicology***

Objective(s):

The phytoestrogen genistein is a primary component of a high soy diet. There is currently widespread interest in the impact of a high soy diet on human health. While there is some indication that phytoestrogens may improve health in peri- and postmenopausal women, there is concern that these compounds may have the potential to be carcinogens. The study is being conducted to investigate this possibility. Specific goals for the project are:

1) To determine the toxicity of genistein in the C57Bl6/J strain and to select doses for the 36-week studies.
2) To identify the potential carcinogenicity of genistein in the p53 transgenic mouse model.
3) To determine if the potential carcinogenicity of genistein relates to changes in the rates of cell death and cell proliferation.
4) To determine if exposure to genistein results in an increase in the mutant frequency in a reporter gene, (*Hprt*), in the splenic lymphocytes of the p53 mouse.

FY 2000 Accomplishments:

1) Protocol approved in FY 2000.
2) Chemical ordered and analyzed by the Division of Chemistry, NCTR.
3) Start-up meeting held to initiate study.

FY 2001 Plans:

1) Conduct the 14-day dose-range-finding study.
2) Initiate 9-month chronic study.

- ***ILSI/HESI Consortium on Application of Genomics and Proteomics to Mechanism-Based Risk Assessment*** — ***P00425*** — ***CDER*** — ***Knowledge Bases***

Objective(s):

1) To establish a database for genomics data.
2) To relate the changes in gene expression to *in vitro* genotoxicity measures that are utilized in hazard assessment

FY 2000 Accomplishments:

A "working group" at the NCTR has been established in order to conduct the experiments for the ILSI project. Initial studies on the extraction of RNA and DNA from *TK*6 cells have been conducted. A dose-range-finding study is nearing completion with the initial chemical, benzo(a)pyrene diol epoxide (BPDE), and experimentation will begin in FY 2001.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Two cell strains will be exposed to known carcinogens, the mutant frequency at the Thymidine kinase locus will be measured, and the formation of specific DNA adducts will be quantified. RNA will be isolated and sent to CDER for gene expression analysis. The data generated from this project will be entered into the ILSI database for further analysis.

◆ ***Methods Development for Chromosome Aberration Analysis by FISH*** — ***X10035*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Chromosomal alterations are associated with the development of tumors and other human diseases. The Division plans to utilize the analysis of chromosomal damage in future research studies. This project would be used to acquire the skills and to establish chromosome analysis techniques for use by the Division. One of the techniques to be established is fluorescence *in situ* hybridization (FISH).

FY 2000 Accomplishments:

New project for FY 2001.

FY 2001 Plans:

1) Training to learn FISH techniques applicable to the evaluation of large-scale genomic damage.
2) Determine the feasibility of developing and implementing the analysis of *TK* mutant clones by FISH analysis.

PI: Parsons, Barbara

◆ ***Measurement of H-ras Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection*** — ***E0704101*** — ***None*** — ***Predictive Toxicology***

Objective(s):

The majority of methods for quantitating mutation require the use of selective drugs that allow mutants to grow and prevent normal cells from growing. All of these techniques require extensive cell culture and can be time-consuming and expensive. There are techniques that utilize genotypic selection that allow for a molecular amplification of the rare mutant DNA sequences and thus provide for a direct measurement of mutant frequencies. The Division has several projects in which this new technology is being developed. The specific goals of this project are:

1) To quantify somatic mutations in liver DNA of mice treated with 4-aminobiphenyl in order to establish and evaluate MutEx/ACB-PCR genotypic selection as an approach for human risk assessment.

2) To determine whether or not the MutEx/ACB-PCR genotypic selection is sensitive enough to measure the spontaneous frequencies of H-*ras* codon 61 CAA AAA mutation in three different mouse models: $B_6C_3F_1$, C57BL/6, and the PMS2 mismatch repair-deficient, transgenic mouse.

FY 2000 Accomplishments:

1) Presented a poster at the Society of Toxicology Meeting in March 2000.
2) A PNA enrichment method for the isolation of mouse H-*ras* sequence has been developed in which biotin-labeled PNA is annealed to H-*ras* sequence and then immobilized and recovered from stretavidin-agarose beads.
3) H-*ras* DNA populations isolated by PNA enrichment were successfully analyzed by MutEx/ACB-PCR. Using this approach, spontaneous H-*ras* codon 61 CAA to AAA mutation was measured in mouse liver genomic DNA.
4) A newborn mouse assay in which $B_6C_3F_1$mice were treated with 4-aminobiphenyl or DMSO (control) was completed, as were dose-range-finding studies in C57BL/6 and PMS2 mice.

FY 2001 Plans:

1) To submit manuscripts describing:
   a) The PNA enrichment method.
   b) An analysis of H-*ras* codon 61 spontaneous mutation in different strains of mice as determined by PNA enrichment and MutEx/ACB-PCR.
   c) The prospects for using genotypic selection methods as a tool for cancer risk assessment (review article).
2) To complete the 4-aminobiphenyl newborn mouse assays in C57BL/6 and PMS2 ($^{+/+}$, $^{+/-}$, and $^{-/-}$) mice.
3) To analyze the liver DNAs of the 4-aminobiphenyl-treated and untreated C57BL/6, $B_6C_3F_1$ and PMS2 mice for H-*ras* codon 61 mutation using PNA selection coupled with MutEx/ACB-PCR.

PI: Pipkin, James

◆ ***The Effect of p53 Null Phenotype on Bleomycin-induced Stress Protein Elicitation In Vivo in Transgenic Mice*** — *E0694901* — *None* — ***Predictive Toxicology***

Objective(s):

1) To investigate the structure of the stress protein (SP) 70 and 90 genes by Southern blot in the eight-to-ten-week-old p53 null mouse in comparison with C57BL/6 control mouse.
2) To investigate the stress protein metabolic turnover (synthesis 35S-labeling) as a reflection of gene expression in the control homozygous C57BL/6 ($^{+/+}$) and the null p53 homozygous TSG ($^{-/-}$) mice as elicited by bleomycin (BL) at 1, 2, 3, 4 and 5 months of age (during the G1-phase of the cell cycle) by polyacrylamide gel electrophoresis (PAGE), and their levels of radio-labeling calculated by

computerized electronic area measurements. If stress proteins (SPS) are absent in bone marrow nuclei of one-month-old p53 null mice (SP synthesis is dependent on the presence of the p53 gene) or if their expression is below the level of measurement, then the protocol will be discontined at test group 1, see below.

3) To investigate the phosphorylation patterns of SPS as a reflection of gene expression as elicited by BL using the same animal types, time-frames, and techniques as in Objective 1.
4) To identify and examine nuclear polypeptides other than SPS for synthesis and phosphorylation levels as possible biomarkers of metabolic alterations and gene expression during phases of the cell cycle in control and homozygous p53 null mice following administration of BL.

FY 2000 Accomplishments:

The research for this project has been completed.

FY 2001 Plans:

Data will be analyzed and the results prepared for publication.

◆ ***ADDEND: The Effect of p53 Null Phenotype on Bleomycin-induced Stress Protein Elicitation In Vivo in Transgenic Mice*** — ***E0694911*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Requesting an additional 16 male pups in order to investigate the assessment of stress protein metabolism phenomenon in two-week-old male pups.

FY 2000 Accomplishments:

The research for this project has been completed.

FY 2001 Plans:

Data will be analyzed and the results prepared for publication.

PI: Shaddock, Joseph

◆ ***ADDEND: Lymphocyte Hprt Mutant Frequencies and Types of Mutations in PMS2 Mice Treated as Neonates with Solvent or with 4-aminobiphenyl*** — ***E0704131*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Because cancer is a disease requiring the induction of mutation and the clonal expansion of mutated cells, one would expect that the developing fetus and young infant would be particularly susceptible to carcinogen exposure. This project provides information that can be used to evaluate this hypothesis. Experiments will be conducted to quantify and identify the *Hprt* mutations in spleen lymphocytes of $PMS2^{+/+}$, $PMS2^{+/-}$, and $PMS2^{-/-}$ mice treated as neonates with either dimethlysulfoxide (solvent control) or with 4-aminobiphenyl (4-ABP).

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) An addendum to protocol E0704101 was written and approved. This addendum involves the quantification and characterization of *Hprt* mutations in mismatch repair-deficient, PMS2 mice treated with 4-aminobiphenyl.
2) Collection of *Hprt* mutant frequencies and mutant sequencing began.

FY 2001 Plans:

The analyses of *Hprt* mutant frequencies in 4-aminobiphenyl treated and control PMS2 $^{+/+}$, $^{+/-}$, and $^{-/-}$ mice will be completed. The characterization of mutant clones will continue until the mutational spectra can be compared between treated and control animals, among animals with different PMS2 genotype, and presumably different DNA repair capacity.

PI: Sheehan, Daniel

◆ ***Development of a Statistically Robust 3D-QSAR Model to Predict In Vitro Rat Uterine Estrogen Receptor Binding Activity*** — ***E0290001*** — ***None*** — ***Knowledge Bases***

Objective(s):

The primary purpose of this CRADA is the development and validation of a statistically robust model for prediction of isolated rat uterine estrogen receptor relative binding affinity (RBA) that could be used as part of a prioritization scheme to identify chemicals for further *in vitro*/*in vivo* screening tests.

FY 2000 Accomplishments:

Approximately 150 chemicals were assayed for estrogen receptor (ER) binding (in replicate) by competition of tritiated estradiol binding. Six papers were published or submitted, including ER binding data for 230 chemicals, a number of computational modeling papers, as well as a comparison of three different methods for quantitating binding activity.

FY 2001 Plans:

We will be working under the Environmental Protection Agency (EPA) Interagency Agreement (IAG) to deliver predictions to the EPA for the binding of 57,000 chemicals to the ER. Additionally, EPA will fund a contract laboratory to assay about 500 chemicals so false positive and negative rates can be estimated. We will train them in the assay technique and calculate the error rates. There will be a limited need for laboratory technical support to assay chemicals for ER binding. These may include: some necessary to have certain structural groups better represented in the models; chemicals whose predicted activity is significantly different from the measured binding; those which we find are impure or unstable; or chemicals whose assayed or predicted values are challenged by other scientists.

Title	Project Number	Collaborator	Strategic Research Goal

- ***ADDEND: Task Order #724 (EPA-IAG Phase 1 (E0290001) Development, Evaluation and Validation of Estrogen Receptor (ER) Models*** — ***E0290011*** — ***None*** — ***Knowledge Bases***

Objective(s):

This task involves the refinement, validation and application of the Estrogen Receptor models for identifying chemicals that bind to the human estrogen receptor and predicting their relative binding affinity to set priorities for EPA.

FY 2000 Accomplishments:

Approximately 150 chemicals were assayed for ER binding (in replicate) by competition of tritiated estradiol binding. Six papers were published or submitted, including ER binding data for 230 chemicals, a number of computational modeling papers, as well as a comparison of three different methods for quantitating binding activity.

FY 2001 Plans:

Working under the EPA IAG scientists will deliver predictions to the EPA for the binding of 57,000 chemicals to the ER. Additionally, EPA will fund a contract laboratory to assay about 500 chemicals so false positive and negative rates can be estimated. The staff will train them in the assay technique and calculate the error rates. There will be a limited need for laboratory technical support to assay chemicals for ER binding. These may include: some necessary to have certain structural groups better represented in the models; chemicals whose predicted activity is significantly different from the measured binding; those which we find are impure or unstable; or chemicals whose assayed or predicted values are challenged by other scientists.

- ***Development of a Statistically Robust Rat Androgen Receptor (AR) 3D-QSAR Model for Predicting Relative Binding Affinity (RBA) of Untested Chemicals*** — ***E0290101*** — ***None*** — ***Concept-Driven***

Objective(s):

To develop and validate a statistically robust 3D-QSAR model to predict *in vitro* rat androgen receptor (AR) relative binding. Provide an alternative and/or supplemental method to prioritize chemicals for entry into Tier 1 screening under the EPA's screening and testing program for endocrine disruptors.

FY 2000 Accomplishments:

Two different assays for binding of chemicals to the androgen receptor (AR) were evaluated. One utilized prostates from castrated rats as the source of a crude preparation (cytosol) of the AR. The results showed high non-specific binding that appeared to mask AR binding, as the dissociation constants were too high for the AR. A number of experimental approaches failed to resolve this problem. Subsequently, an assay using a much purer, commercially available AR proved to give reproducible and valid results. This assay is also less expensive than the prostate assay. In protein

dilution experiments, the dissociation constant provided guidance for the final selection of assay conditions. Based on the validation results, work was started on assaying competition by known AR ligands with tritiated R-1881 as the ligand.

FY 2001 Plans:

The Division will assay approximately 85 chemicals in replicate using a competitive binding assay with tritiated R-1881. These data will be used to develop initial computational models similar to those developed for the ER. Further chemicals will be assayed as needed to provide structural diversity. This will provide the most robust and predictive final computational models.

◆ ***ADDEND: Task Order #725 - EPA IAG Phase 2 - (E0290101) Priority Setting – Androgen Receptor (AR) Binding*** — ***E0290111*** — ***None*** — ***Knowledge Bases***

Objective(s):

To develop, refine, validate and apply the AR models for identifying chemicals that bind to the human androgen receptor and predicting their relative binding affinity to set priorities for EPA.

FY 2000 Accomplishments:

Two different assays for binding of chemicals to the androgen receptor (AR) were evaluated. One utilized prostates from castrated rats as the source of a crude preparation (cytosol) of the AR. The results showed high non-specific binding that appeared to mask AR binding, as the dissociation constants were too high for the AR. A number of experimental approaches failed to resolve this problem. Subsequently, an assay using a much purer, commercially available AR proved to give reproducible and valid results. This assay is also less expensive than the prostate assay. In protein dilution experiments, the dissociation constant provided guidance for the final selection of assay conditions. Based on the validation results, work was started on assaying competition by known AR ligands with tritiated R-1881 as the ligand.

FY 2001 Plans:

We will assay approximately 85 chemicals in replicate using a competitive binding assay with tritiated R-1881. These data will be used to develop initial computational models similar to those developed for the ER. Further chemicals will be assayed as needed to provide structural diversity. This will provide the most robust and predictive final computational models.

PI: Valentine, Carrie

◆ ***Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the FX174 am3*** — ***E0697701*** — ***None*** — ***Predictive Toxicology***

Title	Project Number	Collaborator	Strategic Research Goal

Objective(s):

One of the *in vivo* models being developed by DGRT contains a *FX174* transgene. This project addresses the characterization and evaluation of this assay for hazard identification. The specific goals of this project are:

1) To establish the experimental parameters necessary to demonstrate a mutant frequency of 1.5- to 2-fold above background.
2) To establish the sensitivity of the am3 mouse model to mutagenic carcinogens and germ-cell mutagens expected to produce DNA damage at A:T base pairs.
3) To compare, where possible, the sensitivity of the ϕX174 system with that of other *in vivo* mutational systems.
4) To establish several basic properties of the ϕX174 am3 assay by determining the tissue or organ specificity of responses to certain carcinogens and by determining the patterns of mutations detected by the assay.

FY 2000 Accomplishments:

1) Treat 87 transgenic ΦX 174 mice with ethyl nitrosourea (ENU) at different doses, times, and control solvents.
2) Collected tissue from all and did *Hprt* assay on all. Submitted the technical report to close.

FY 2001 Plans:

None.

◆ ***ADDEND: Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the FX174 am3*** — ***E0697711*** — ***None*** — ***Predictive Toxicology***

Objective(s):

Addendum requesting a minor addition to the protocol to bring a more conclusive and precise evaluation of the ϕX174 transgenic mouse system. An additional experiment will help us find out if DNA from non-replicating cells may lead to the lower sensitivity in the ϕX174 assay. Requesting three pregnant ϕX174 transgenic mice.

FY 2000 Accomplishments:

The technical report was submitted on 9/14/00.

FY 2001 Plans:

None.

Title	Project Number	Collaborator	Strategic Research Goal

- ***The Development of Transgenic Mice Harboring Bacteriophage ΦX174 with Sites Specific for Detecting Mutations at Guanosine:Cytosine Nucleotides, Small Frameshifts, and Extended Deletions*** — ***E0700201*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To find specific mutations in bacteriophage ΦX174 that render the bacteriophage non-infectious and that will revert to plaque-forming ability only when mutation occurs by specific mechanisms: 1) base substitution at a G:C base pair, or 2) frameshift caused by deletion of one or two nucleotides. An additional objective is to determine the feasibility of using ϕX174 to detect the deletion of an extended sequence. Phage harboring these mutations will be used to construct a transgenic mouse model for measuring mutations *in vivo.*

FY 2000 Accomplishments:

1) Manuscript published.
2) Forward assay fully developed for ΦX174 mouse.
3) Target sites and mutant spectrum for solvent and ENU-treated cells identified for ΦX 174 Forward assay.
4) License offered for Forward assay.

FY 2001 Plans:

Research for this project has been completed and the data will be prepared for publication.

- ***Automated Mutational Analysis of the PhiX174 Forward Assay*** — ***X10032*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To develop a protocol for an automated analysis of the PhiX174 Forward mutation assay. The automation of the assay would provide for a rapid method to detect induced mutations. Most of the *in vivo* mutation assays require extensive cell culture and long times to detect and evaluate the induced mutant frequency. The PhiX174 Forward assay is being developed to provide for a more rapid analysis.

FY 2000 Accomplishments:

Summer student showed that a microplate assay for counting mutant plaques is feasible.

FY 2001 Plans:

Develop protocol and submit for approval. Initial experimentation will focus on the improvement of the microplate assay to widen the difference between negative and positive samples. Begin development of microsphere assay for sequencing mutants.

FY 2000 Publications

Banfalvi, G., Littlefield, N.A., Hass, B.S., Mikhailova, M.V., Csuka, L. and Chou, M.W., Effect of Cadmium on the Relationship Between Replicative and Repair DNA Synthesis in Synchronized CHO Cells, European Journal of Biochemistry, 267:6580-6585. Accepted: 9/8/2000 **(E0696701)**.

Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R.G. and Sheehan, D.M., The Estrogen Receptor Binding Affinities of 188 Natural and Xenochemicals: Structural Diversity of Ligands, Toxicological Sciences, 54:138-153. Accepted: 11/3/1999 **(E0700801)**.

Casciano, D.A., Development and Utilization of Primary Hepatocyte Culture Systems to Evaluate Metabolism, DNA Binding and DNA Repair of Xenobiotics, Drug Metabolism Reviews, 32(1):1-13. Accepted: 6/1/2000 **(Z9999942)**.

Desai, V.G., Aidoo, A., Li, J., Lyn-Cook, L.E., Casciano, D.A. and Feuers, R.J., Effects of Bleomycin on Liver Antioxidant Enzymes and the Electron Transport System from *Ad Libitum* and Dietary Restricted Female and Male Fischer 344 Rats, Nutrition and Cancer, 36(1):42-51. Accepted: 10/1/1999 **(E0699101)**.

Dobrovolsky, V.N., Shaddock, J.G. and Heflich, R.H., 7,12-dimethylbenz[a]anthracene-Induced Mutation in the *tk* gene of $TK^{+/-}$ Mice, Environmental and Molecular Mutagenesis, 36:283-291. Accepted: 6/17/2000 **(E0701801)**.

Fang, H., Tong, W., Perkins, R.G. and Sheehan, D.M., Quantitative Comparisons of *In Vitro* Assays for Estrogenic Activities, Environmental Health Perspectives, 108:723-729. Accepted: 5/21/2000 **(P00385)**.

Feuers, R.J., Desai, V.G., Chen, F., Hunter, J., Duffy, P.H. and Oriaku, E.T., Effects of Dietary Restriction on Insulin Resistance in Obese Mice, AGE, 23:101-107. Accepted: 5/18/2000 **(E0699801)**.

Hansen, D.K., Laborde, J.B., Wall, K.S., Hinson, W.G., Pipkin, J.L., Shaddock, J.G., Lyn-Cook, L.E. and Young, J.F., Dose-Response of Retinoic Acid Induced Stress Protein Synthesis and Teratogenesis in Mice, Reproductive Toxicology, 15(1):31-41. Accepted: 9/22/2000 **(E0697601)**.

Heddle, J.A. and Casciano, D.A., *In Vivo* Transgenic Mutation Assays, Environmental and Molecular Mutagenesis, 35:253-259. Accepted: 1/5/2000 **(NA)**.

Heflich, R.H. and Casciano, D.A., *In Vivo* Gene Mutation Assays and Their Application in Cancer Risk Assessment, Mutation Research Newsletter. Accepted: 4/14/2000 **(E0690601)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Hinson, W.G., Pipkin, J.L., Wu, J. and Young, J.F., Using Visualization to Confirm Mathematical Analysis, Proceedings of the 3rd International Workshop on Intelligent Control and Systems, Atlantic City, NJ. Accepted: 2/1/2000 **(NA)**.

Khaidakov, M., Study on Genotoxic Effects of the Space Environment: A Comparison Between Experienced Cosmonauts and Unexposed Russian Twins, Mutation Research, 430:337-342. Accepted: 10/15/1999 **(NA)**.

Lu, M., Tang, N. and Ali, S.F., Effect of Single Injection of Methylazoxymethanol at Postnatal Day One on Cell Proliferation in Different Brain Regions of Male Rats, Neurotoxicology. Accepted: 6/19/2000 **(P00406)**.

MacGregor, J., Casciano, D.A. and Muller, L., Strategies and Testing Methods for Identifying Mutagenic Risks, Mutation Research, 455:3-20. Accepted: 9/30/2000 **(Z9999905)**.

Manjanatha, M., Shelton, S.D., Culp, S.J., Blankenship, L. and Casciano, D.A., DNA Adduct Formation and Molecular Analysis of *In Vivo LacI* Mutations in the Mammary Tissue of Big Blue Rats Treated with 7,12-dimethylbenz[a]anthracene, Carcinogenesis, 21:265-273. Accepted: 10/8/1999 **(E0690611)**.

Mittelstaedt, R.A., Smith, B.A., Beland, F.A. and Heflich, R.H., Sequence Specificity of *Hprt* Lymphocyte Mutation in Rats Fed the Hepatocarcinogen 2-acetylaminofluorene, Mutation Research, 431:167-173. Accepted: 10/5/1999 **(E0697021)**.

Sheehan, D.M., Activity of Environmentally Relevant Low Doses of Endocrine Disruptors and the Bisphenol A Controversy: Initial Results Confirmed, Proc Soc Exp Biol Med, 224(2):57-60. Accepted: 6/17/2000 **(NA)**.

Shelton, S.D., Cherry, V.R. and Manjanatha, M., Mutant Frequency and Molecular Analysis of *In Vivo lacI* Mutations in the Bone Marrow of Big Blue (R) Rats Treated with 7,12-dimethylbenz[a]anthracene, Environmental and Molecular Mutagenesis. Accepted: 6/25/2000 **(E0690601)**.

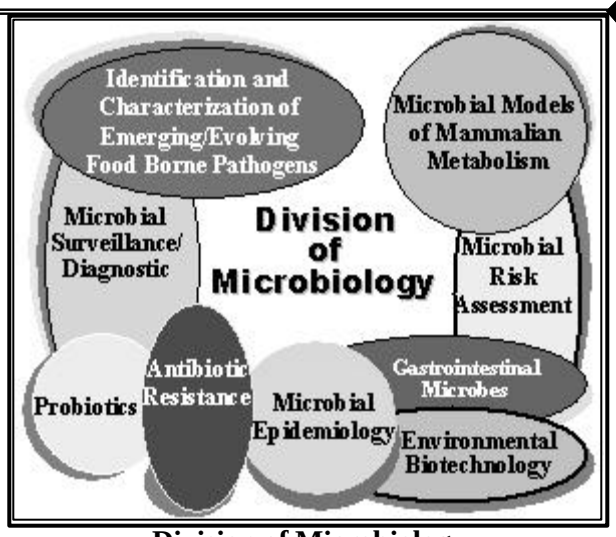
Tu, M., Tong, W., Perkins, R.G. and Valentine, C.R., Predicted Changes in Pre-mRNA Secondary Structure Vary in Their Association with Exon Skipping for Mutations in Exons 2, 4, and 8 of the *Hprt* Gene and Exon 51 of the Fibrillin Gene, Mutation Research, 432:15-32. Accepted: 10/15/1999 **(E0700211)**.

Turturro, A., Hass, B.S. and Hart, R.W., Does Caloric Restriction Induce Hormesis?, Human and Ecological Risk Assessment, 19:320-329. Accepted: 1/1/2000 **(E0050300)**.

MICROBIOLOGY

Director: ***Carl E. Cerniglia, Ph.D.***
Telephone: **870-543-7341**
Toll Free: **800-638-3321**
E-mail: **ccerniglia@nctr.fda.gov**

Executive Summary



Division of Microbiology Research Initiatives

The Division of Microbiology serves a multipurpose function for the FDA/NCTR with specialized expertise to perform fundamental and applied microbiology research in areas of toxicology. The Division of Microbiology also responds to microbial surveillance and diagnostic needs for research projects. Projects are selected based on FDA priorities and programmatic expertise. The research program is divided into five focal areas: 1) foodborne pathogens, food safety and methods development; 2) gastrointestinal microbiology and host interactions; 3) environmental biotechnology; 4) the use of microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals; and 5) microbiological surveillance and diagnostic support of research.

In FY 2000, research scientists within the Division provided valuable information to FDA to evaluate key regulatory issues in food safety, with special emphasis on antimicrobial resistance in the food animal production environment.

Reports of antibiotic-resistant bacteria from farms and animal carcasses are raising concerns that antibiotic use in agriculture may play a role in selecting for antibiotic resistance. This is a very controversial issue, since some contend that the indiscriminate use of antibiotics in agriculture could create a massive reservoir of resistant microorganisms in the environment that could infect humans through the food chain. However, others contend that the abuse of antibiotics in human medicine may instead be largely responsible for the increase in antibiotic resistance. Animal drug industry representatives feel that there is not enough evidence to conclusively demonstrate a link between the use of antibiotics in food animals and the emergence of antibiotic-resistant bacteria. The research and regulatory issues on antimicrobials used in food-producing animals are of great importance to the FDA.

As part of the Food Safety Initiative, the Division of Microbiology established collaborative research agreements with scientists at the Center for Veterinary Medicine (CVM), Arkansas Poultry and Livestock Commission, and the Department of Poultry Sciences, University of Arkansas. Researchers in the Division of Microbiology have collected litter, feed and water samples from farms to isolate *Salmonella* and *Campylobacter* to determine if they are fluoroquinolone-resistant. Our studies indicate

that poultry products may serve as reservoirs of fluoroquinolone-resistant *Campylobacter* spp.

Microbiologists in the Division have developed a multiplex polymerase chain reaction (PCR) method that is rapid and sensitive for the detection of *Salmonella typhimurium* DT104 from clinical, food, and environmental samples. Currently, this method is being used by the Office of Regulatory Affairs field laboratories to detect *S. typhimurium* DT104 in food samples.

Since there has been concern about the use of antibiotics in agriculture, other approaches are being evaluated to minimize contamination of animal products with foodborne human pathogens. Reducing colonization of animals by pathogenic bacteria by using competitive exclusion treatments, phage therapy, vaccines, and farm hygiene is being considered as an alternative to antimicrobial feed additives.

Competitive exclusion products must adhere to FDA regulations that the bacterial mixtures be well defined. For commercial use, competitive exclusion preparations for poultry must be free from all known human and avian pathogens and from any microorganisms with unusually high resistance to antimicrobials. The FDA has approved a competitive exclusion product, Preempt^{TM}, designed to prevent the colonization of chicken intestines by pathogenic bacteria, such as *Salmonella* spp., *Campylobacter* spp., and *Escherichia coli*, and also to reduce the use of antibiotics and the spread of antibiotic-resistance genes. The manufacturer reports that the product contains 29 well-defined isolates of facultatively and obligately anaerobic bacteria. However, the Division has found several discrepancies between conventional identification and the list of components reported. The results indicate that FDA will need to standardize the identification techniques used to characterize the components of competitive exclusion products. In addition, researchers have determined that lactobacilli in Preempt^{TM} contain a vancomycin-resistance gene. A multiplex PCR was developed in FY 2000 to simultaneously amplify all the markers for vancomycin resistance in any bacterial strain.

In response to FDA's need for assessing the microbiological safety of animal drug residues in food, the Division and the CVM have been performing pre-validation studies on an *in vitro* system that examines the effect of low-level antibiotic residues on the human intestinal microflora by using a chemostat to model the human intestinal tract.

Another essential study in the Division is the elucidation of the mechanism of resistance to antimicrobial agents among bacteria from the human gastrointestinal tract. The resistant bacteria are of particular concern, because not only do they act as a reservoir for antimicrobial resistance genes, but also if they get out of place and establish themselves in other parts of the body, they can cause diseases that cannot be treated.

The environmental fate of veterinary drugs and the factors that influence the persistence and biodegradation of antibiotics used in farm animals have been investigated. Both fundamental and applied studies on the biodegradation pathways of veterinary antimicrobial drugs have been conducted.

A unique resource for FDA in the Division of Microbiology is the Surveillance/Diagnostic Program with microbiological support services for the pathogen-free animals used for research at NCTR. This service includes monitoring the health status of rodents, rabbits, and non-human primates. The Surveillance/Diagnostic Program ensured that infections and infectious diseases did not bias any animal experiments at NCTR in FY 2000.

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Campbell, Warren

- ***Microbiological Diagnostic Methods: Development, Testing and Evaluation*** — ***E0026200*** — ***None*** — ***Method-Driven***

  Objective(s):

  To improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology, and serology as applicable to NCTR programs and projects.

  FY 2000 Accomplishments:

  Developed PCR assay for *Helicobacter hepaticus*.

  FY 2001 Plans:

  1) Develop polymerase chain reaction (PCR) assay for *Helicobacter bilis.*
  2) Develop procedure for immunosuppressive screening of animals for pathogens using animal isolators in building 14-A containment laboratory.

- ***General Microbiological Support – Bacteriology, Parasitology, Mycology, and Virology*** — ***S00006*** — ***None*** — ***Center Support***

  Objective(s):

  To provide the Center with microbiological surveillance and diagnostic support to determine and maintain the health status of the Animal Colony.

  FY 2000 Accomplishments:

  Performed routine surveillance evaluation on the following: 1,036 animals; 122 animal waste; 5,339 cage waters; 470 room air; 7,322 room swabs; 282 processed feed; 92 processed water; 11 feed shipment; 7,254 biological indicators for sterility; and 97 personnel surveillance.

  FY 2001 Plans:

  Plan to routinely complete the following evaluations: 1,036 animals; 122 animal waste; 5,339 cage waters; 470 room air; 7,322 room swabs; 282 processed feed; 92 processed water; 11 feed shipment; 7,254 biological indicators for sterility; and 97 personnel surveillance.

- ***Special Epidemiology Investigations of Potential Microbiological Contamination Problems*** — ***S00185*** — ***None*** — ***Center Support***

  Objective(s):

  1) To investigate potential microbiological contamination problems.
  2) To report non-routine sample time which is not recorded on Sample Collection Report (SCR).

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

Conducted a special epidemiological investigation of building 5-A automated water system and found the source of *Pseudomonas aeruginosa* contamination.

FY 2001 Plans:

Conduct a major screening of NCTR animal breeding colony for *Helicobacter hepaticus*.

PI: Erickson, Bruce

◆ ***An In Vitro System to Study Effects of Low Dose Antimicrobials on the Human Intestinal Microflora***	***X00051***	***None***	***Knowledge Bases***

Objective(s):

To use an *in vitro* chemostat culture system that mimics the human intestinal tract to determine the concentrations of antibiotic residues in food that produce no adverse effect on the human intestinal microflora. The adverse effects to be evaluated include: 1) changes in numbers of selected organisms; 2) changes in the metabolic activity of the fecal flora relating to metabolism of endogenous and exogenous compounds; 3) development of antimicrobial-resistant strains; and 4) disruption of colonization resistance of pathogenic microorganisms.

FY 2000 Accomplishments:

1) Antibiotics are used both therapeutically and as growth promoters in animals destined for human consumption. Residues of the antibiotics may remain in the animal tissues when they are brought to market. A pre-validation study was conducted on a method to test the effect on the human intestinal microflora, of low level antibiotic residues in food.
2) Using a series of chemostats to model the bacterial population of the human large intestine, selected bacterial species were monitored for an increase in antibiotic resistance upon the addition of different levels of ciprofloxacin.
3) The normal population of bacteria in the human intestines serves as a natural barrier to colonization of the gut by pathogenic bacteria. Using the chemostat system, tested the effect of low levels of ciprofloxacin on the ability of the normal intestinal microflora to resist colonization by a *Salmonella* species.
4) The results of our ciprofloxacin studies were presented to an international task force on microbial safety, where both the *in vitro* and an *in vivo* method for addressing the issue of antibiotic residues in food were evaluated.

FY 2001 Plans:

1) Submit a research protocol through the NCTR protocol review and approval system for continued work using the chemostat system to measure the effect of low-level antibiotic residues on the human intestinal microflora.

2) Continue analysis of the samples collected during exposure of the chemostats to ciprofloxacin, examining metabolic enzyme activity, volatile fatty acid concentrations, and bile acid metabolism as alternative endpoints for system perturbation by low-level antibiotic residues.
3) Using the information and experience gained during the ciprofloxacin experiment, initiate a new chemostat experiment for testing a veterinary fluoroquinolone antibiotic such as enrofloxacin or danofloxacin.
4) Prepare a topic paper on normal variations in human intestinal microflora for the microbial safety task force, which will be used to recommend procedures for preparing fecal inocula for *in vitro* or *in vivo* testing.

PI: Khan, Ashraf

◆ ***Studies on Mechanism of Fluoroquinolones Resistant Salmonella spp. Isolated from Animal Feeds (Poultry), Animal Production Environment and the Development of Molecular Methods for Screening the Drug Resistance Genes*** — ***E0704801*** — ***CVM*** — ***Method-Driven***

Objective(s):

1) To isolate, identify and characterize nalidixic acid and fluoroquinolone-resistant *Salmonella* spp. from chicken farms (animal feed, feces, manure, litters, and animals) by biochemical and polymerase chain reaction.
2) To determine minimum inhibitory concentration for environmental isolates, development of molecular techniques and its comparison with clinical strains.
3) To determine drug-resistance mechanisms in the environmental isolates and their characterization by molecular techniques.
4) To determine influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella* spp.

FY 2000 Accomplishments:

1) Developed multiplex PCR method for the rapid detection of multidrug-resistant *Salmonella typhimurium*.
2) Developed mismatch amplification mutation assay for the detection of ciprofloxacin-resistant *Salmonella* spp.
3) Isolated several nalidixic acid (antibiotic)-resistant *E. coli* from chicken and turkey farms.
4) Characterized fluoroquinolones-resistant *Campylobacter* spp. from chicken and turkey farms by pulse-field gel electrophoresis.

FY 2001 Plans:

1) Isolation and molecular characterization of fluoroquinolone-resistant *Salmonella* spp. and *E. coli* from chicken and turkey litters.

Title	Project Number	Collaborator	Strategic Research Goal

2) Determine the influence of climatic conditions on the occurrence of quinolone-resistant *Salmonella* spp. and *E. coli.*

PI: Khan, Saeed

◆ ***Molecular Screening Methods for the Determination of Vancomycin Resistance in Selective Competitive Exclusion Product CF3 (Preempt™) Bacteria*** — ***E0705301*** — ***None*** — ***Method-Driven***

Objective(s):

1) To islolate, identify, and biochemically characterize vancomycin-resistant bacteria present in a commercially available competitive exclusion product CF3.
2) To develop a rapid PCR method of the detection of vancomycin-resistance determinant genes, namely, the *VanA0, VanB, VanC* and D-ala-D-lac ligase gene Ddl.
3) To characterize plasmid DNA Profile and plasmid-mediated drug resistance transfer.
4) To conduct genetic fingerprinting of the vancomycin-resistant microorganisms present in an Preempt™ culture.
5) To conduct nucleotide sequence analysis of the PCR products of vancomycin-resistant determinant genes showing interesting restriction profiles.

FY 2000 Accomplishments:

1) Ten vancomycin-resistant bacteria belonging to genus lactobacillus were isolated from the competitive exclusion (CE) product Preempt™.
2) Developed individual PCR assays for the detection of *VanA*, *VanB*, *VanC1*, *VanC2* and *VanC3* genes in ATCC control strains.
3) Developed multiplex-PCR assay to simultaneously detect *VanA*, *VanB*, *VanC1*, *VanC2* and *VanC3* genes in a single-reaction tube. This provides a fast detection procedure and also cuts down the cost of reagents used.
4) Developed PCR assay for the detection of *ddlA/B* genes in lactobacilli.

FY 2001 Plans:

1) PCR products of vancomycin-resistance determinant genes will be digested with restriction enzymes and if different restriction profiles are obtained for the same gene, the nucleotide sequence analysis of the variants will be carried out.
2) Study the plasmid profile of vancomycin-resistant bacteria isolated from the Competitive Exclusion Product. If any plasmids are detected, their ability to transfer the resistance markers to enterococcal, lactobacillus, and staphylococcal strains of the poultry and human origin will be tested.
3) Genetic fingerprinting and strain typing of vancomycin-resistant organisms isolated from the CE product will be carried out by pulse-field gel electrophoresis and molecular probing.

Title	Project Number	Collaborator	Strategic Research Goal

4) A collaborative research protocol with the FDA CVM on "The fate and degradation of antimicrobials, oxytetracycline (OTC), sulfadimethoxine-ormeto-prim (Romet-30) from aquaculture environmental samples" will be initiated.

PI: Nawaz, Mohamed

- ***Studies on the Fluoroquinolone Resistance in Campylobacter spp. Isolated from Poultry*** — ***E0705001*** — ***CVM*** — ***Method-Driven***

Objective(s):

1) To isolate and identify fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli* from water, feed, and litter samples in poultry houses.
2) To determine the optimum concentration of nalidixic acid and fluoroquinolone resistance in *C. jejuni* and *C. coli.*
3) To determine the influence of various seasons and the frequency of isolation of fluoroquinolone-resistant *C. jejuni* and *C. coli.*
4) To conduct molecular characterization of fluoroquinolone resistance by polymerase chain reaction (PCR), nucleotide sequencing, and single-strand conformation polymorphism (SSCP).

FY 2000 Accomplishments:

1) Determined that chicken harbors fluoroquinolone antibiotic-resistant *Campylobacter* spp. These drug-resistant bacteria were exclusively present in "chicken liver" samples.
2) Determined that fresh turkey litter contains fluoroquinolone-resistant *Campylobacters*.
3) Found that all fluoroquinolone-resistant *Campylobacters* were resistant to at least five different antibiotics.
4) Found that the antibiotic-resistant profiles of chicken and turkey isolates were different.
5) Standardized a pulse-field gel electrophoresis (PFGE) method to characterize these isolates at the molecular level.
6) Found that a PCR-RFLP of flagellin gene indicate at least six different groups of *Campylobacters*.

FY 2001 Plans:

1) Complete and send results to CVM for review and prepare subsequent manuscripts for publications.
2) Collect more isolates for epidemiological studies.
3) Correlate the environmental data with the occurrence of *Campylobacters*.
4) Characterize all isolates at the molecular level (PCR-RFLP, PFGE).
5) Correlate the environmental data (season/month) to the occurrence of *Campylobacters*.

Title	Project Number	Collaborator	Strategic Research Goal

PI: Pothuluri, Jairaj

- ***Microbial Degradation of Drugs and Feed Additives Used in Fish Farming (Aquaculture)*** — *E0690101* — *CVM* — *Method-Driven*

Objective(s):

1) To develop a standardized method to evaluate the biodegradation of drugs and feed additives used in fish farming (aquaculture).
2) To determine the biodegradation rates and metabolic fate of the antibiotic erythromycin in aquaculture water and sediments.

FY 2000 Accomplishments:

Microbial degradation of antibiotic erythromycin was studied using NCTR microcosm test system that mimics natural environment.

FY 2001 Plans:

A project completion report will be submitted.

- ***Fungal and Mammalian-Metabolism of Selected Pesticides Used in Food Productions for Human Consumption*** — *X90014* — *None* — *Knowledge Bases*

Objective(s):

1) To determine the biotransformation of hazardous organophosphorus insecticides, parathion and methyl-parathion, and phenylurea-like herbicides, monuron and diuron, using selected a group of fungi with known biotransformation potential.
2) To isolate and identify the metabolites of these pesticides and elucidate the metabolic pathway(s).
3) To compare the mammalian metabolism of these compounds with fungal metabolism and ascertain the risk associated with the use of these compounds to human health and/or environmental safety.

FY 2000 Accomplishments:

Not applicable.

FY 2001 Plans:

Suggested starting date – 4/1/2001.

PI: Rafii, Fatemeh

- ***Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds*** — *E0700701* — *None* — *Concept-Driven*

Objective(s):

1) To detect various metabolites of phytoestrogens, produced by the metabolism of these compounds by pure culture of bacteria typical of that isolated from human

microflora, and elucidation of the metabolic pathways of phytoestrogens by human intestinal bacteria.

2) To assess the estrogenic effect of each phytoestrogen metabolite produced by intestinal bacteria.
3) To determine the bacterial species producing estrogenic metabolites from phytoestrogens and elucidation of enzymes involved in various steps of these metabolic processes.
4) To determine the effects of phytoestrogens and their metabolites on the population, composition, metabolic activity, and enzyme production of bacteria from the human gastrointestinal tract.

FY 2000 Accomplishments:

1) Bacterial species from the human intestinal tract that are involved in the conversion of daidzin and genistein to more estrogenic compounds, daidzein and genistein, were detected.
2) Intestinal anaerobic bacteria with demethylating enzymes were used to produce estrogenic metabolites from natural isoflavonoids.
3) Anaerobic bacteria from the human intestinal tract involved in the reductive metabolism of daidzein and genistein were identified.

FY 2001 Plans:

1) Detect specific bacteria from the human intestinal tract involved in the conversion of phytoestrogens (daidzein and genistein) to estrogenic and nonestrogenic end products.
2) Evaluate the effect of fluoroquinolones on resistance development in anaerobic bacteria from the human intestinal tract, mechanism of resistance development, impact on metabolic activities, and the dissemination of resistance to bacterial pathogens.
3) Develop resistance to nitro antimicrobial drugs in bacteria from the human intestinal tract and evaluation of the role of reductive enzymes in resistance development.

PI: Sutherland, John

◆ ***Biotransformation of Fluoroquinolones by Fungi*** — ***E0705201*** — ***None*** — ***Method-Driven***

Objective(s):

1) To measure the kinetics of biodegradation of veterinary fluoroquinolone drugs in natural matrices.
2) To identify the potential metabolites produced by fungi from fluoroquinolones.
3) To assess the residual antibacterial activity and potential risks of the metabolites formed from these drugs.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) Three fungi were shown to transform a model phenothiazine drug, *N*-acetylphenothiazine, to *N*-acetylphenothiazine sulfoxide, phenothiazine sulfoxide, phenothiazin-3-one, phenothiazine *N*-glucoside, and phenothiazine.
2) The fungus *Mucor ramannianus* was shown to transform the veterinary fluoroquinolone drug enrofloxacin to enrofloxacin *N*-oxide, *N*-acetylciprofloxacin, and desethylene-enrofloxacin.
3) Methods for preparing food samples for the chromatographic analysis of flavors and off-flavors in foods were reviewed.
4) The mycotoxin fusarin C was shown not to have a role in the formation of DNA adducts by *Fusarium* culture extracts.

FY 2001 Plans:

1) Studies on metabolism of the veterinary fluoroquinolone sarafloxacin by *Mucor ramannianus* and other fungi.
2) Studies on the method of formation of unusual hydroxyvinylcyclopentenone conjugates from ciprofloxacin and norfloxacin by the fungus *Trichoderma viride*.
3) Identification of the metabolites produced from norfloxacin and sarafloxacin by *Trichoderma viride* grown on rice hulls (poultry litter).
4) Isolation of new strains of fungi from litter in poultry houses and screening them for the biotransformation of veterinary fluoroquinolones.

PI: Wagner, Robert

- ***In Vitro Model and Molecular Analysis of Competitive Exclusion Products*** — ***E0704901*** — ***CVM*** — ***Method-Driven***

Objective(s):

1) To evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 and CRL-2117 cell monolayers.
2) To define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements.
3) To sequence analysis of 16s rRNA polymerase chain reaction (PCR) products from defined culture component bacteria and development of a database containing the sequences for use in subsequent identification of the organisms in undefined CE products.
4) To apply the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.

FY 2000 Accomplishments:

1) Completed *in vitro* assay of competitive exclusion product.
2) Identified 18 of 27 bacterial isolates from Preempt™ by multiple phenotypic and genotypic techniques.

Title	Project Number	Collaborator	Strategic Research Goal

3) Evaluated 18 of 27 MIC analyses from Preempt™ isolates.

FY 2001 Plans:

1) Complete the objectives and send results to CVM for review.
2) Propose a new project of antibiotic residue effects on colonization resistance.
3) Propose an *in vivo* assay of probiotic effects on host defense.

FY 2000 Publications

Bever, R.J., Couch, L.H., Sutherland, J.B., Williams, A.J., Beger, R., Churchwell, M.I., Doerge, D.R. and Howard, P., DNA Adduct Formation by *Fusarium* Culture Extracts: Lack of Role for Fusarin C., Chemico-Biological Interactions, 128:141-157. Accepted: 8/2/2000 **(E0211101)**.

Cerniglia, C.E., Shuttleworth, K.L., Methods for Isolation of Polycyclic Aromatic Hydrocarbon (PAH)-Degrading Microorganisms and Procedures for Determination of Biodegradation Intermediates and Environmental Monitoring of PAHs, Manual of Environmental Microbiology, 2nd Edition. Accepted: 9/12/2000 **(E0678700)**.

Cerniglia, C.E., Recent Advances in the Biodegradation of Polycyclic Aromatic Hydrocarbons by Mycobacterium Species, NATO ASI Series. Accepted: 7/13/2000 **(E0699901)**.

Cerniglia, C.E., The JECFA and Alternate Approaches for Determining ADIs for Antimicrobial Residues, Microbial Ecology in Health and Disease, Suppl 1:30-34. Accepted: 8/8/2000 **(NA)**.

Cho, B.P., Blankenship, L., Moody, J.D., Doerge, D.R., Beland, F.A. and Culp, S.J., Synthesis and Characterization of 4'-amino and 4'-nitro Derivatives of 4-N,N-Dimethylaminotriphenylmethane as Precursors for a Proximate Malachite Green Metabolite, Tetrahedron, 56:7379-7388. Accepted: 7/22/2000 **(E0212801)**.

Greenlees, K. and Cerniglia, C.E., Lincomycin, WHO Technical Report Series, 45:3-38. Accepted: 1/24/2000 **(NA)**.

Holland, R.D., Rafii, F., Heinze, T.M., Sutherland, J.B., Voorhees, K.J. and Lay, J.O., MALDI TOF/MS Detection of Bacterial Biomarker Proteins Isolated from Contaminated Water, Lettuce, and Cotton Cloth, Rapid Communications in Mass Spectrometry, 14:911-917. Accepted: 3/21/2000 **(E0700501)**.

Hur, H. and Rafii, F., Biotransformation of the Isoflavonoids Biochanin A, Formononetin, and Glycitein by Eubacterium Limosum, FEMS Microbiology Letters, 192:21-25. Accepted: 8/30/2000 **(E0700701)**.

Hur, H., Lay, J.O., Beger, R., Freeman, J.P. and Rafii, F., Isolation of Human Intestinal Bacteria Metabolizing the Natural Isoflavone Glycosides Daidzin and Genistein, Archives of Microbiology, 174:422-428. Accepted: 9/26/2000 **(E0700701)**.

Khan, A.A., Nawaz, M.S., Khan, S.A. and Cerniglia, C.E., Detection of Multidrug-Resistant *Salmonella Typhimurium* Dt104 by Multiplex Polymerase Chain Reaction, FEMS Microbiology Letters, 182 (2):355-360. Accepted: 11/18/1999 **(E0704801)**.

Project Number Codes:

E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Khan, S.A., Nawaz, M.S., Khan, A.A. and Cerniglia, C.E., Transfer of Erythromycin Resistance from Poultry to Human Clinical Strains of *Staphylococcus aureus*, Journal of Clinical Microbiology, 38:1832-1838. Accepted: 2/26/2000 **(E0690101)**.

Moody, J.D., Heinze, T.M. and Cerniglia, C.E., Fungal Transformation of the Tricyclic Antidepressant Amoxapine: Identification of N-carbomethoxy Compounds Formed as Artifacts by Phosgene in Chloroform Used in the Extraction of Metabolites, Biocatalysis and Biotransformation. Accepted: 9/26/2000 **(E0690101)**.

Moody, J.D., Zhang, D., Heinze, T.M. and Cerniglia, C.E., Transformation of Amoxapine by *Cunninghamella elegans*, Applied Environmental Microbiology, 66:3646-3649. Accepted: 5/16/2000 **(E0694201)**.

Nawaz, M.S., Khan, S.A., Khan, A.A., Khambaty, F.M. and Cerniglia, C.E., Comparative Molecular Analysis of Erythromycin-Resistance Determinants in *Staphylococcal* Isolates of Poultry and Human Origin, Molecular and Cellular Probes, 14:311-319. Accepted: 8/26/2000 **(E0690101)**.

Parshikov, I., Freeman, J.P., Lay, J.O., Beger, R., Williams, A.J. and Sutherland, J.B., Microbiological Transformation of Enrofloxacin by the Fungus *Mucor ramannianus*, Applied and Environmental Microbiology, 66:2664-2667. Accepted: 3/21/2000 **(E0705201)**.

Shuttleworth, K.L., Kim, E. and Cerniglia, C.E., Physiological and Genetic Comparisons of Two Aromatic Hydrocarbon-Degrading *Sphingomonas* Strains, Molecules and Cells, 10:199-205. Accepted: 2/11/2000 **(E0699901)**.

Sietmann, R., Moody, J.D. and Cerniglia, C.E., Hydroxylation of Biphenyl by the Yeast *Trichosporon mucoides*, Archives of Microbiology, 174:353-361. Accepted: 9/8/2000 **(NA)**.

Wagner, R.D. and Balish, E., Probiotic Effects of Feeding Heat-Killed *Lactobacillus acidophilus* and *Lactobacillus casei* to *Candida albicans*-Colonized Immunodeficient Mice, Journal of Food Protection, 63:638-644. Accepted: 12/5/1999 **(NA)**.

Wagner, R.D., Effects of Probiotic Bacteria on Humoral Immunity to *Candida albicans* in Immunodeficient bg/bg-nu/nu and bg/bg-nu/+ mice, Rev Iberoam Micol., 17:55-59. Accepted: 5/5/2000 **(NA)**.

Wang, R., Cao, W., Khan, A.A. and Cerniglia, C.E., Cloning, Sequencing, and Expression in *Escherichia Coli* of a Cytochrome P450 Gene from *Cunninghamella elegans*, FEMS Microbiology Letters, 188:55-61. Accepted: 5/8/2000 **(E0694201)**.

Project Number Codes:

E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Wang, R., Myers, M.J., Campbell, W.L., Cao, W., Paine, D.D. and Cerniglia, C.E., A Rapid Method for PCR Detection of Bovine Materials in Animal Feedstuffs, Molecular and Cellular Probes, 14:1-5. Accepted: 10/5/1999 **(P00408)**.

Wang, R., Wennerstrom, D.E., Cao, W., Khan, A.A. and Cerniglia, C.E., Cloning, Expression, and Characterization of the katG gene, Encoding Catalase-Peroxidase, from the Polycyclic Aromatic Hydrocarbon-Degrading Bacterium *Mycobacterium* sp. strain PYR-1, Applied and Environmental Microbiology, 66:4300-4304. Accepted: 8/4/2000 **(E0699901)**.

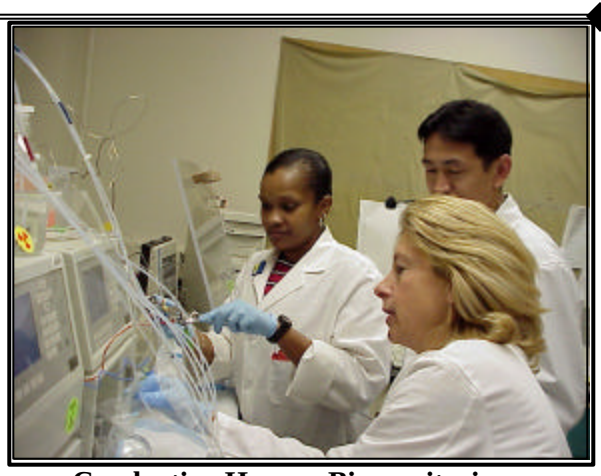
Wilkes, J.G., Conte, E.D., Kim, Y., Holcomb, M., Sutherland, J.B. and Miller, D.W., Sample Preparation for the Analysis of Flavors and Off-Flavors in Foods, Journal of Chromatography A – Special Issue, 880:3-33. Accepted: 2/28/2000 **(E0693101)**.

Project Number Codes:

E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

MOLECULAR EPIDEMIOLOGY

Director: ***Fred F. Kadlubar, Ph.D.***
Telephone: **870-543-7204**
Toll Free: **800-638-3321**
E-mail: **fkadlubar@nctr.fda.gov**



Conducting Human Biomonitoring Experiments

Executive Summary

The strategic goals of the Division of Molecular Epidemiology are: 1) the identification of genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy; 2) the conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices; 3) human exposure biomonitoring and DNA adduct detection; 4) the extrapolation of the results of animal bioassays and of mechanistic studies to humans; and 5) the development and validation of DNA Microarray Technology for human diagnostics.

The intent is to better understand the mechanisms of human carcinogenesis; to provide an estimation of human exposure to direct and indirect-acting carcinogens; to assess the importance of inter-individual differences in carcinogen and drug bioactivation, detoxification, or induced changes in gene expression; and to suggest intervention strategies for human cancer prevention. Accordingly, Division research has provided new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. Our research has been focused on the foodborne heterocyclic amines, environmental aromatic amines and polycyclic aromatic hydrocarbons, on widely used drugs including selected benzodiazepines, antihistamines, drugs inducing peroxisomal proliferation or oxidative stress, on estrogens, antiestrogens and endocrine disruptors, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, larynx, breast, ovary, prostate, lung, urinary bladder, and bone marrow are ongoing. These are outlined as follows:

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

1. Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.
   a) Genetic and epigenetic regulation of cytochrome P450 1A2.
   b) Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression.
   c) Polymorphisms of sulfotransferases.
   d) Polymorphisms of glutathione S-transferases.
   e) Inter-individual variation in DNA repair capacity.
   f) Characterization of peroxidases toward metabolic activation.
   g) Gender-specific variation in drug metabolism.

2. Chemoprevention.
   a) Modulation of expression of multi-drug-resistance genes.
   b) Coffee and tea effects on *N*-acetyltransferases.
   c) Effects of tea constituents on expression of genes cytochrome P450 1A2, H-*ras*, and the normal epithelial cell specific gene (NES1).
   d) DNA methylation, DNA methyltransferases, and homocysteine toxicity.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices:

1. Etiology of human colorectal cancer: role of dietary heterocyclic amines.
2. Etiology of human breast and prostate cancers in African-Americans.
3. Etiology of human pancreatic cancer: role of carcinogen and drug exposures, chronic pancreatitis, and dietary imbalance.

Human exposure biomonitoring and DNA adduct detection:

Biomarkers of exposure and susceptibility for breast, prostate, laryngeal, lung, colon, and urinary bladder cancers.

Extrapolation of the results of animal bioassays and of mechanistic studies to humans:

Evaluation of the neonatal mouse bioassay as an alternative bioassay for selected benzodiazepines, antihistamines, chloral hydrate, drugs inducing peroxisomal proliferation or oxidative stress, synthetic and natural estrogens, and endocrine disruptors, including chlorinated hydrocarbon pesticides and dinitroaniline herbicides (See Biochemical Toxicology Executive Summary.)

International efforts in molecular epidemiology and biotechnology:

1. Organization of the Molecular Epidemiology Group of the American Association for Cancer Research.
2. Development and validation of "DNA Microarray Technology" for assessing individual risk for cancer susceptibility and recurrence, adverse drug reactions, and therapeutic drug efficacy.

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Coles, Brian

◆ ***Dietary Isothiocyanates, Glutathione S-transferases, and Colorectal Neoplasia*** — ***E0320001*** — ***None*** — ***Concept-Driven***

Objective(s):

To explore the relationship between dietary isothiocyanates, glutathione *S*-transferase induction, and colon polyp recurrence. NCTR's direct objective is to quantitate glutathione *S*-transferase in human plasma.

FY 2000 Accomplishments:

1) Preparation of approved protocol/grant.
2) Preparation and approval of Cooperative Research and Development Agreement (CRADA) to allow transfer of designated funds to NCTR.
3) Validation of methodology to be used: i.e., use of hGSTA1 antibody assay as the appropriate methodology to measure GST alpha in plasma; the finding that GSH affinity matrices and HPLC are not quantitative when using plasma (cf. work with other tissues).
4) Seminar invitation to Arizona Cancer Center.

FY 2001 Plans:

1) Employment of technician (CRADA-supported) to assist with project.
2) Quantitation of GST alpha in approximately 600 plasma samples, 20% in duplicate to assess accuracy.
3) Determination of hGSTM1 and hGSTT1 genotypes with albumin as control, approximately 600 genomic DNA corresponding to the plasma samples.
4) Preparation of report for Arizona Cancer Center, with discussion and preparation of manuscripts as appropriate.

PI: Hammons, George

◆ ***Methylation Profile, Gene Expression, and Enzyme Activity of CYP1A2 in Human Livers*** — ***E0696201*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To determine the possible involvement of epigenetic mechanisms in the regulation of the expression of the *CYP1A2* gene. The methylation status determined for each sample will be correlated with the expression of the *CYP1A2* gene and enzyme activity.

FY 2000 Accomplishments:

Mechanistic analysis examining the methylation status of the CpG site adjacent to an AP-1 binding site in the 5'-flanking region of *CYPlA2* has provided important insight into its regulation. The mutation state was found to vary among individuals and

Project Number Codes:
E–Ongoing P–Preliminary S–Support X–Proposed Project/Concept Paper

hypermethylation of this site appears to be associated with reduced *CYPlA2* expression. Mechanistic data on the regulation of *CYPlA2* is critical in predicting drug efficacy and safety in individuals and individual susceptibility to carcinogenic agents.

FY 2001 Plans:

Mechanistic analysis in *CYPlA2* regulation will be continued by examining additional specific CpG sites in *CYPlA2*. Our approach will utilize methylation-specific PCR. The sample pool will also be increased.

PI: Kadlubar, Fred

◆ ***Rapid, Population-based, Screening Methodology for Genetic Polymorphisms in Adverse Drug Metabolizing and/or Cancer-Related Risk Alleles*** — ***E0300001*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To develop and fabricate a SNP microarray chip or "risk-tox chip" for the analysis of genetic polymorphisms that affect individual cancer or adverse drug risk.
2) To validate the "risk-tox chip" by comparative analyses with standardized methodologies.
3) To automate the methodologies for large population risk assessment using the "risk-tox chip" in a robotic work station.
4) To establish NCTR as an alpha test site to introduce "risk tox chip" screening analysis as rapid and reliable frontline screening methodology for clinical and population-based molecular epidemiological studies.

FY 2000 Accomplishments:

The Division established and validated conventional genotyping methods for 28 gene targets and their associated polymorphisms. First attempts at DNA microarray chips have proven to be a very reliable for profiling of genotypes as demonstrated by a more than 99% concordance between the microarrays and conventional genotyping assays (PCR-RFLP), based on a mini-chip made so far (*ras, NAT2*, and *COMT*).

In a colon polyp prevention trial involving 1,429 subjects, questionnaire information was used to assess potential exposure to heterocyclic amines. Using our high-throughput DNA microarrays, 686 individuals who had undergone colonoscopy by year three were genotyped for all common *NAT2* alleles in one work-day. Only those in the highest tertile of red meat consumption who were rapid acetylators showed a significant increased risk and the odds ratios indicated gene-dose dependence. These data suggest that a one-third reduction in red meat consumption is in itself a sufficient preventive measure for colon polyp recurrence and thus should appreciably lower colorectal cancer risk.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

To achieve our goals, staff are developing a DNA microarray platform to genotype patients for all the major enzyme variants that would enable us to predict carcinogen susceptibility, adverse drug reactions, and perhaps chemotherapeutic drug efficacy. VistaMorph Arrays, which are low-/medium-density microarrays formatted for very high throughput genotyping applications currently allow up to 250 oligonucleotide probes per array to be configured to interrogate single nucleotide polymorphisms (SNPs) or polymorphisms due to gene deletion or SNPS across hundreds of samples per day in a fully automated production facility. DNA genotyping microarray technology provides a means of rapid, large-scale data/risk mining in population, and it will impact pharmacogenomics, pharmacogenetics, as well as SNPs associated with cancer susceptibility. At present, the VistaMorph Arrays are being fabricated and will include *NAT1, NAT2, COMT, CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, GSTA1, GSTA2, GSTM1, GSTM3, GSTT1, GSTP1, GSTZ1, SULT1A1, MTHFR, NQO1, MPO, SOD, FMO3, HYL, UGT1A1, UGT1A6, UGT2B7, UGT2B7,* and *K-ras*. In FY 200l, staff will also complete implementation of our conventional genotyping assays and carry out validation studies with these VistaMorph Arrays.

◆ ***Chemoprotection of DNA Adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in the Rat*** | ***E0689401*** | ***None*** | ***Predictive Toxicology***

Objective(s):

To examine the effect of the glutathione S-transferase inducers, phenethylisothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tert-butyl-4-hydroxyanisole (BHA), kahweol palmitate, cafestol palmitate, quercetin, tannic acid, α-angelicalactone, Green tea, and ethoxyquin on the metabolism and DNA adduct formation of the food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, in the Fischer 344 rat.

FY 2000 Accomplishments:

Preliminary studies with human hepatocytes were carried out with the coffee lipids, kahweol and cafestol, which had been shown to down-regulate N-acetyltransferase in rat hepatocytes. However, the experiments were unsuccessful due to apparent toxicity of the compounds.

FY 2001 Plans:

This experiment needs to be repeated before the project is completed. Then, a manuscript can be submitted for publication and final report prepared.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***A Case-Control Study of Pancreatic Cancer and Aromatic Amines***	***E0694601***	***None***	***Predictive Toxicology***

Objective(s):

To measure the associations of aromatic amine exposure and metabolism with the risk of pancreatic cancer. The sources of aromatic and heterocyclic amines to be studied are cigarette smoking and diet; the metabolic capabilities to be studied are acetylator status and N-oxidation status.

FY 2000 Accomplishments:

1) Analysis of molecular epidemiological data from our completed case-control study on pancreatic cancer. Data indicate that the slow *NAT1*4* allele is a significant risk factor.
2) Laboratory studies on chronic pancreatitis, which is the strongest predisposing factor for the development of pancreatic cancer, has shown a 5-to-15-fold increase in the levels of *CYP1A1, CYP1B1, CYP2C9*, and *CYP3A4*, with the latter present at the highest levels, comparable to about 5-10% of that found in human liver.
3) Variation in expression of GST phenotype has been assessed, with hGSTA2, known to be critical for carcinogen detoxification and protection from lipid peroxidation, being the major isoform in normal pancreas and found to be strongly down-regulated in pancreatitis.
4) A novel GST polymorphism in the coding region of hGSTA2 was also discovered and found to modify activity 2.5-fold.
5) Three publications were submitted and accepted during this period.

FY 2001 Plans:

The case-control studies are being further analyzed and, together with the laboratory studies, will be prepared for publication in FY 2001.

◆ ***Role of Acetylation and N-Oxidation in Colorectal Cancer***	***E0694701***	***None***	***Predictive Toxicology***

Objective(s):

To confirm the initial findings of our pilot study regarding the roles of heterocyclic amine metabolism and exposure as putative risk factors from the diet or the environment. The sources of heterocyclic amines to be studied are cigarette smoking, diet and cooking methods; the metabolic pathways to be studied include heterocyclic amine *N*-oxidation status and *O*-acetylation status.

FY 2000 Accomplishments:

1) Analysis of molecular epidemiological data from our completed case-control study on colorectal cancer at the UAMS. Dietary questionnaires are being evaluated that will give good exposure estimates to food-borne heterocyclic amines.
2) Laboratory studies have defined new polymorphisms in sulfotransferases and led to a series of publications characterizing these enzyme systems.

3) Discovered a novel polymorphism in the 5'-regulatory region of human glutathione S-transferase Al (hGSTAl), which significantly lowers its expression.
4) In case-control studies in Arkansas (collaboration with the UAMS) and in Arizona (collaboration with the Arizona Cancer Center), the low-activity variant was shown to confer a 2-to-3-fold increased risk for colorectal cancer.

FY 2001 Plans:

The case-control studies are being further analyzed and, together with the laboratory studies, will be prepared for publication in FY 200l.

◆ ***Chemical Carcinogenesis: Epithelial Cells in Breast Milk*** — ***E0697801*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction.
2) To detect and quantify aromatic/hydrophobic-DNA adducts in luminal epithelial cells derived from human breast milk.
3) To detect genetic polymorphisms in carcinogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk.
4) To evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in *NAT1*, *NAT2*, *CYP1A1*, and *GSTM1*.

FY 2000 Accomplishments:

1) Laboratory and animal data indicate that several classes of carcinogens, including aromatic and heterocyclic amines and PAHs, could be etiologic factors in breast cancer. However, epidemiologic studies do not support an association between cigarette smoking, a vehicle for delivery of those carcinogens, and breast cancer risk. This project was designed to develop methodology to separate exfoliated ductal epithelial cells from human breast milk. Ductal epithelial cells are those from which most breast cancers arise, thus, they are the ideal target tissue for evaluation of carcinogen-DNA adducts. In this study, exfoliated cells were isolated and evaluated for DNA adducts. In collaboration with EPA, milk from the same samples was evaluated for mutagenicity in *Salmonella* strain YG1024, which is a frameshift strain derived of TA98 that over expresses the acetyltransferase gene and is highly specific for detecting aromatic and heterocyclic amines. In these studies, DNA adducts were identified in 66% of the specimens. Bulky adducts by TLC appeared to run in a pattern similar to aromatic amine standards, in particular 4-aminobiphenyl, a suspect mammary carcinogen. For milk mutagenicity, an acid hydrolysis method followed by dichloromethane/n-hexane extraction with gel permeation was developed to enrich for aromatic and heterocyclic amine compounds from milk. For samples processed in this fashion (n=25), 88% (22/25) were positive for mutagenic activity. These data, to date: 1) corroborate previous findings of mutagenicity of

human milk; 2) demonstrate the utility of breast fluids, such as milk, as non-invasive sources for identifying the source of mutagen activity; and 3) illustrate the need to develop methods to quantify human exposures to potential carcinogens.

2) A manuscript detailing our results is in preparation and will be submitted for publication in the near future.

FY 2001 Plans:

A large amount of effort has been expended in development of extraction methodologies for mutagenicity assays to identify the carcinogens to which human breast epithelial cells are exposed. While we have implicated carcinogen-DNA adducts in human breast milk, the nature of the putative adducts has not been elucidated. Because we are collecting extensive exposure data from women who are providing milk specimens, our ultimate aim is to evaluate associations between adducts, mutagenicity, and exposures to chemical carcinogens, as well as modification of relationships by genetic polymorphisms in genes related to carcinogen metabolism. Prior to that analysis, however, a primary goal is to identify the carcinogens in milk and those responsible for DNA adducts. In an effort to achieve these goals, we will further examine the nature of the DNA adducts present by using ^{32}P-postlabelling-HPLC in the presence of synthetic standards. We will also utilize a recently developed neutral extraction procedure that is amenable to analysis by LC/MS in an attempt to identify 4-aminobiphenyl and other DNA-damaging agents that may be present in breast milk.

◆ ***The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis*** | ***E0699001*** | ***None*** | ***Predictive Toxicology***

Objective(s):

To elucidate the role of human cytochrome P450 IB1 (*CYP1B1*) in drug metabolism and carcinogenesis. Specific aims are:

1) To design and develop peptide-specific antibodies against human *CYP1B1*.
2) To determine the levels of *CYP1B1* protein in various human tissues.
3) To evaluate *CYP1B1* expression as a biomarker for tumorigenesis.
4) To identify *CYP1B1* inducers among the most common drugs and carcinogens.
5) To identify *CYP1B1* substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes *CYP1A1* and *CYP1A2*.
6) To find specific enzyme inhibitors for *CYP1B1*.
7) To develop a sensitive, convenient, and specific assay method for *CYP1B1* enzyme activity *in vitro*.
8) To evaluate genetic polymorphism(s) for *CYP1B1* as an epidemiological marker for cancer risk.

FY 2000 Accomplishments:

1) Project completed.
2) Manuscripts in press.
3) Final report in preparation.

FY 2001 Plans:

None planned.

◆ ***ADDEND: The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis*** — ***E0699011*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To add *in situ* hybridization as an additional approach to investigate the expression of *CYP1B1* in various human tissues. This was performed in addition to the immunohistochemistry of the protocol. Requesting inclusion of Pathology support in the performance of these studies.

FY 2000 Accomplishments:

1) Project completed.
2) Manuscript published.
3) Final report in preparation.

FY 2001 Plans:

None planned.

◆ ***In Vivo Modeling of Steroid-mediated Gender Effects in Drug Metabolism*** — ***E0704301*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To characterize the activity of *CYP1A2* in female subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT.
2) To characterize the activity of *CYP1A2* in male subjects with regard to age.
3) To measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6, and IL-10 levels in female and male subjects studied for *CYP1A2* activity.
4) To correlate the activity of *CYP1A2* with circulating levels of cytokines and/or circulating levels of steroid hormones.
5) To statistically assess the impact of each of the measured variables on the *CYP1A2* phenotype.

FY 2000 Accomplishments:

Phase I (E0704301) of the initial study required the recruitment of 160 participants divided into eight subgroups for assessment of hormone and cytokine levels and *CYP1A2* activity. Phase II (E0704311), which received further funding in 1999, added recruitment of a ninth subgroup, another 20 pregnant volunteers, to the overall set of participants and added technical support to assess hormone and cytokine

interactions with CYPs 3A and 2D6. Because two probe drugs were being administered simultaneously, validation of lack of interaction of the two drugs was done by repeating a portion of the initial volunteers.

Before initiation of Phase II of the study, four-repeat series from 25 volunteers had been completed in Phase I (E0704301 used caffeine only as probe drug). Of these 25, eleven individuals reproduced the full four-series three times, taking the probe drug caffeine only (original Phase I data), dextromethorphan only (added probe of Phase II), and the combination drug regime to validate lack of drug-interaction with the two probe drugs.

Thus, a total of 47 sets of four repeat-series (188 total samples) have been collected. IL-6 assays and hormone measures (estradiol, progesterone, cortisol and testosterone) using plasma/serum samples and *CYP1A2* analyses using urine samples from these series have been completed. The initial data showed for the first time that constitutive levels of *CYP1A2* appear to be controlled by levels of IL-6 in women and varies during the menstrual cycle.

FY 2001 Plans:

IL-l and IL-10 assays have not yet been completed on this original sample set. These assays should be completed early in the year. This will complete all laboratory analyses planned for these samples. Recruitment for E0704301 is now terminated. As indicated, a third of the participants re-consented and repeated the study. Additionally, those volunteers who were enrolled in the database but had not yet begun in the study, were re-contacted and re-consented for study E0704311.

◆ ***ADDEND: Part II of In Vivo Modeling of Steroid-mediated Gender-effects in Drug Metabolism*** | ***E0704311*** | ***None*** | ***Predictive Toxicology***

Objective(s):

1) To determine the activity of *CYP2D6* and 3A4 in female and male subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT.
2) To measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for CYP activity.
3) To correlate the activity of *CYP2D6* and 3A4 with circulating levels of cytokines and/or circulating levels of steroid hormones.
4) To statistically assess the impact of each of the measured variables on the *CYP2D6* phenotype and *CYP3A4* activity level.

FY 2000 Accomplishments:

Sample processing and recruitment is proceeding at a successful rate in most subgroups. Ten individuals are registered in the database waiting to start the study. In one year under this study we have successfully recruited 34% of the targeted study

subjects for analysis and completed sample processing on 80% of those samples collected to date.

We are currently seeking a new consultant in the UAMS, Department of Obstetrics and Gynecology. Efforts have also been made to improve recruitment of individuals in older subgroups through the Center on Aging, UAMS, which is lending support in the recruitment effort.

A total of 223 samples has been collected from 62 individuals. IL-6 assays are complete on all female samples collected to date and will soon be completed on all males as well. *CYPlA2* activity is currently being determined on all samples.

FY 2001 Plans:

IL-6, IL-1 and IL-I0 assays and *CYP1A2* analysis are planned for this sample set as recruitment continues. To date, 80% of the specimens collected have been analyzed for IL-6, hormones, and *CYP1A2*. IL-l and IL-10 assays will begin in 2001. Recruitment for E0704311 will continue with additional efforts to increase participation of individuals over 55 years of age.

PI: Lyn-Cook, Beverly

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***The Effects of Nicotine and Other Cigarette Components on Normal and Neoplastic Human Pancreatic Cells: The Role of Low Zinc Levels on Ras, mdr-1 Genes Activation and Metabolizing Enzyme Activities as a Possible Risk Factor for Pancreatic Cancer***	***E0701701***	***None***	***Predictive Toxicology***

Objective(s):

1) To determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*.
2) To examine *ras, mdr-1, CYP1A1* and *CYP1A2* expression in normal and neoplastic human pancreatic tissue grouped according to race and sex obtained from a human tissue bank.

FY 2000 Accomplishments:

1) Nicotine exerts gender differences on expression of critical genes involved in carcinogenesis, metabolism, and toxicity in normal and tumorigenic pancreatic cells.
2) *CYP1A2* and *DT-diaphorase* are highly expressed in human pancreatic tumor cell lines and preliminary studies show high expression in human pancreatic tissue from heavy smokers and pancreatic adenocarcinomas.
3) *CYP1A1* was not expressed in pancreatic tumor cells.
4) Nicotine up-regulates *NF-kB, K-ras, Nes-I*, and *Mn SOD* in pancreatic cells. HnRNP was up-regulated in pancreatic tumor cells from males. Nicotine

addictivity in cigarettes has been proposed as a regulatory issue for FDA. FDA regulates transdermal nicotine patches used in smoking cessation therapy. This study seeks to determine if FDA should be concerned about nicotine's long-term effects on critical genes involved in carcinogenesis and as a potential risk factor for the development of pancreatic cancer.

FY 2001 Plans:

1) Complete *in vitro* studies on zinc effects on nicotine-treated cells.
2) Conduct study on zinc effects on expression of metabolic gene expression (*CYP1A2* and DT-diaphorase) and on activity.
3) Increase the number of human pancreatic tissues from cancer patients and from smokers, particularly specimens from African-Americans.
4) Complete studies on cotinine and nornicotine (nicotine metabolites) in relation to their effects on normal and pancreatic tumor cells.

◆ ***ADDEND: Colorectal Adenoma Study - H-ras and K-ras Methylation (Task 4)*** ***E0707131*** ***None*** ***Predictive Toxicology***

Objective(s):

To provide analytical support for the analysis of H-*ras* and K-*ras* methylation in red blood cell specimens for an intramural study being conducted by the Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI).

FY 2000 Accomplishments:

Interagency Agreement (IAG) was approved.

FY 2001 Plans:

This protocol seeks to develop better biological assays to predict human genetic damage and potential mechanisms of action of chemopreventive agents in pancreatic cancer.

◆ ***ADDEND: Colorectal Adenoma Study - IGF-1 Hypermethylation (Task 5)*** ***E0707141*** ***None*** ***Predictive Toxicology***

Objective(s):

To provide analytical support for the analysis of IGF-1 hypermethylation in red blood cell specimens for an intramural study being conducted by the Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI).

FY 2000 Accomplishments:

IAG was approved.

FY 2001 Plans:

To develop better biological assays to predict human genetic damage and potential mechanisms of action of chemopreventive agents in pancreatic cancer.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***CYP1B1 Expression in Human Brain Tumors: A Potential Role for Estrogen as a Neuroprotective Agent***	***X00052***	***None***	***Concept-Driven***

Objective(s):

To define the role of human cytochrome *CYP1Bl* in drug metabolism and carcinogenesis.

FY 2000 Accomplishments:

CYP 1B1 was found to be highly expressed in human brain tumor cells lines (SK-N and SK-NS). Preliminary work showed that an HPLC method could be developed to detect metabolites of 17-beta estradiol in these cells. Initial work further demonstrated that in addition to *CYP1B1* expression, *CYP1A1*, AhR and ARNT are highly expressed in these cells.

FY 2001 Plans:

Submit Master protocol:

1) Determine the effects of specific neurosteroids (Pregnenolone and DHEA) on estrogen metabolism in brain cells.
2) Examine the effects of estrogen and its metabolites on cell proliferation, apoptosis, enzyme activity- *CYP1Bl*, *CYP1A1*, AhR ARNT, and aromatase expression.
3) Determine the effects of Premarin on modulation of metabolic enzymes in brain cells.

PI: Poirier, Lionel

◆ ***Methylation Status and Cancer Risk***	***E0704601***	***None***	***Predictive Toxicology***

Objective(s):

To learn whether methylation status, determined by noninvasive procedures, may be a biomarker of cancer risk in humans. The methylation status will be assessed by measurement of SAM, SAH and homocysteine in blood, and of DNA hypomethylation in lymphocytes. Two-thirds of the work will be supported under the terms of an IAG from the National Cancer Institute (NCI).

FY 2000 Accomplishments:

The major scientific findings made in this study are the following:

1) Dietary homocystine increases blood homocysteine and produces a hypomethylating environment and increased hyperplasia *in vivo.*
2) The elevated activity of DNA methyltransferase in preneoplastic liver is partly the result of the presence of endogenous, competing DNA in the nuclear preparation of the enzyme.
3) Alterations in methyl metabolism previously seen in cancer have been extended to diabetes and clinical depression in humans and to therosclerosis in rats.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) Bring to a stage of reasonable evaluation the blood analyses of SAM, SAH and HCys in two large clinical collaborative studies: one on COMT and breast cancer and the other on the effects of rapid weight loss on several biomarkers of health.
2) Compare the responses to enzyme inhibitors by the DNA methyltransferases in normal and neoplastic colon.
3) Complete studies on diabetes, depression, and atherosclerosis.
4) Examine changes in specific site methylation in preneoplastic livers in a collaborative study with the National Cancer Center Research Institute (NCCRI) of Japan.

◆ ***Colorectal Adenoma Study - Task 1*** ***E0707101*** ***None*** ***Predictive Toxicology***

Objective(s):

To provide analytical support for the analysis of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

FY 2000 Accomplishments:

Finalized IAG and shipped tissue samples.

FY 2001 Plans:

The FDA/NCTR will measure SAM and SAH concentrations on 521 (467 study subjects plus 54 quality control samples) blood specimens from the previous colorectal adenoma study undertaken by NCI.

◆ ***ADDEND: Colorectal Adenoma Study - SAM and SAH (Additional samples for Task 1)*** ***E0707111*** ***None*** ***Predictive Toxicology***

Objective(s):

To provide analytical support for the analysis of S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

FY 2000 Accomplishments:

IAG was approved.

FY 2001 Plans:

The FDA/NCTR will measure SAM and SAH concentrations on 521 (467 study subjects plus 54 quality control samples) blood specimens from the previous colorectal adenoma study undertaken by NCI.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***ADDEND: Colorectal Adenoma Study (Task 3)***	***E0707121***	***None***	***Predictive Toxicology***

Objective(s):

To provide analytical support for the analysis of MTHFR specific activity in red blood cell specimans for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

FY 2000 Accomplishments:

Finalized IAG and collection of tissue samples.

FY 2001 Plans:

This is a pass-through of funds from the National Cancer Institute (NCI) through the NCTR to the Veteran's Administration in Little Rock. The MTHFR levels in lymphocytes from the same 165 subjects and patients as in E0707111, E0707131, and E0707141 will be determined. The results from the controls and the patients will be compared with each other, with all other methyl-related parameters in the NCI study, as well as with dietary intakes.

PI: Sweeney, Carol

◆ ***Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors***	***E0701501***	***None***	***Method-Driven***

Objective(s):

To examine the role of inter-individual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women. By evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism, the effects of substances regulated by the FDA on breast cancer risk in African-American women may be further elucidated. Additionally, a successful model to increase African-American participation in research studies would greatly assist in future studies related to FDA-regulated substances in African-American populations.

FY 2000 Accomplishments:

1) The above project is a case-control study of genetic and environmental risk factors for breast cancer in African-American and Caucasian women. The purpose of this pilot study was to develop a novel method of recruitment, focused primarily on minority women, and investigate previously unexplored risk factors in breast cancer epidemiology. Eligible cases and controls are contacted by women who are breast cancer survivors and asked to participate in the study. To date, interviews have been completed for 274 women with breast cancer, aged 29-75, and 141 community controls. The participation rate (the proportion of women who complete the study), is 76% for Caucasian women and 61% for African-Americans.

The infrastructure for case-control epidemiologic studies has been built, and a specimen bank was established to enable exploration of future hypotheses.

2) A paper detailing the recruitment strategies, "Challenges, Limitations and Strategies for Increasing Participation in Epidemiologic Studies," was rewritten and has been resubmitted.
3) In the spring of 2000, presentations were made regarding recruitment methodology in our study at a Keystone Conference in Taos entitled, "Molecular Epidemiology: A New Tool in Cancer Prevention."
4) Presented in poster format at the Annual Meeting of the American Association for Cancer Research (AACR).
5) An abstract detailing recruitment methods was also selected for a platform presentation at the 2000 Department of Defense Era of Hope Breast Cancer Research Program Meeting. A collaborative paper using specimens from control subjects is in press.

FY 2001 Plans:

Continue data collection through 2001. Methodologies are in place and currently identifying patients from several hospitals and records from physicians with large breast surgery practices in Little Rock. Interviews are ongoing. When data collection is complete, genotyping for polymorphisms in a number of genes involved in the metabolism of carcinogens and hormones will be performed, and evaluated in relation to case-control status and exposure information derived from questionnaire data. A grant was submitted to NCI (6/00) to expand the study (Racial Patterns in Breast Cancer Etiology and Pathology). If funded, this project will be amended to include collaborations at institutions in Louisiana and Tennessee and expand the geographic breadth of the study so that we may enroll larger numbers of African-American women to explore complex gene/environment interactions.

◆ ***ADDEND: The Role of Glutathione S-transferasse genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy*** | ***E0701511*** | ***None*** | ***Predictive Toxicology***

Objective(s):

1) To determine expression of enzymes (phenotype) in tumor tissue from women who received adjuvant therapy for breast cancer, using biopsy or surgical tissue specimens, using immunohistochemistry, and to evaluate associations between phenotypes in tumor tissue and risk of breast cancer recurrence.
2) To determine inherited GSTM1, GSTT1 and GSTP1 genotypes in normal tissue from these same women, and to determine associations of GSTM1, GSTT1 and GSTP1 genotype with phenotype in tumor tissue.
3) To evaluate if GST genotypes predict breast cancer recurrence following treatment, controlling for other factors that may relate to prognosis.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) This project addresses the role of genetic polymorphisms in enzymes involved in the metabolism of chemotherapeutic agents, as well as reactive oxidant products created by radiation and chemotherapy, in survival after treatment for breast cancer. To date, normal archived tissue has been genotyped for *GSTM1*, *GSTPI*, and *GSTT1*, and relationships between genotype and survival evaluated.
2) A paper reporting the findings for *GSTP1* is in press and another reporting results for GSTM1 and GSTTl is in preparation.
3) Data were also presented at the AACR meeting (4/00) by Carol Sweeney, who received an AACR Susan G. Komen Foundation Young Investigator Scholar Award for the abstract.
4) Interested in the relationship between enzyme expression in tumor tissue and survival, as well as association with genotype. To date, tumor tissue has been stained for GSTP1, GST alpha, and GST mu. However, statistical analyses have not yet been conducted.
5) For 250 women treated for breast cancer, subject eligibility has been determined, follow-up data compiled, archived pathology blocks retrieved, and genotyping assays conducted. Data on concordance of genotypes from paired tumor and normal tissue samples have also been compiled.
6) For 103 subjects, immunohistochemical staining for GSTPl, GST alpha, and GST mu expression was completed and statistical analysis for survival according to *GSTPl* , *GSTMl* , and *GSTTI* genotypes was carried out.
7) Findings on the association between GSTPl variant genotype and survival among women treated for breast cancer were submitted for publication, and a manuscript has been submitted on *GSTMl* and *GSTTI* genotypes and survival.

FY 2001 Plans:

1) Work will continue.
2) Relationships between enzyme expression in tumor tissue and inherited polymorphisms in genes encoding those enzymes will be evaluated.
3) Evaluate associations between tumor tissue levels and survival.
4) Expand the protocol to evaluate additional polymorphisms of interest, including *GSTAl*, *CYP3A4*, and MnSOD.
5) Conduct a small study to evaluate the possibility that the use of tumor tissue for genotyping may produce artifactual results, by comparing genotypes for the GSTs in paired normal and tumor tissue from the same individuals.
6) Determine GST enzyme expression in breast tumor tissue for additional subject samples as well as prepare manuscripts describing: a) concordance of genotype results between paired breast tumor and normal tissue samples; b) correlation between GST genotypes and GST expression in breast cancer; and c) association between GST expression and survival among women treated for breast cancer.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Prostate Cancer: Exposure, Susceptibility and DNA Adducts***	***E0702101***	***None***	***Method-Driven***

Objective(s):

1) To determine levels of carcinogen exposure in African-Americans and Caucasians with histologically confirmed prostate cancer using a case-control design.
2) To evaluate variability in hormone metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen metabolism, and to evaluate the interaction of these factors with the exposure data obtained in 1 above.
3) To characterize DNA adducts in prostate tissue from men with prostate cancer to identify mutagenic agents and to evaluate levels of adducts in relation to carcinogen exposure data and susceptibility factors obtained in 1 and 2 above.

FY 2000 Accomplishments:

Little is known regarding etiologic factors in prostate cancer. Furthermore, it is unclear why African-American men have the highest prostate cancer rates in the world. This project is a case-control study in Arkansas that consists of two projects. The first is a nested study; men who are undergoing a needle biopsy for a high PSA or palpable mass are asked to consent to withdrawal of an additional core. Specimens have been stored for 669 men, 202 of whom have been diagnosed with prostate cancer. If patients are diagnosed with prostate cancer and agree to participate in the case-control study, their tissue will be evaluated for carcinogen-DNA adducts. The larger case-control study ascertains patients from a number of hospitals. To date, 298 cases and 80 controls have been interviewed. Specimens are being banked for genotyping at the completion of data collection.

FY 2001 Plans:

1) Data collection will continue for this study, with cases ascertained and interviewed, and specimens collected.
2) Data entry is being performed on an on-going basis.
3) Begin postlabeling assays to identify DNA adducts in biopsy specimens from men with prostate cancer.
4) Develop a study to utilize the sample set of more than 400 subjects with prostate biopsies who consented to the study and were free of cancer. These will include laboratory methods for measurement of additional markers of DNA damage and mutations in normal tissue from men free of cancer and ascertainment of cancer incidence during two to five years of follow-up among biopsy (cancer)-negative men.

FY 2000 Publications

Agus, C., Ilett, K. and Minchin, R.F., Characterization of ATP-Dependent Pathway of Activation for the Heterocyclic Amine Carcinogen N-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline (N-OH-IQ), Carcinogenesis, 21:1213-1219. Accepted: 1/20/2000 **(E0689421)**.

Ambrosone, C.B., Oxidants and Antioxidants in Breast Cancer (Review), Antioxidants and Redox Signaling. Accepted: 5/20/2000 **(NA)**.

Chou, M.W., Mikhailova, M.V., Nichols, J.A., Poirier, L.A., Warbritton, A.R. and Beland, F.A., Interactive Effects of Methyl-deficiency and Dietary Restriction on Liver Cell Proliferation and Telomerase Activity in Fischer 344 Rats Treated with Aflatoxin B_1, Cancer Letters, 152 (1):53-61. Accepted: 12/1/1999 **(E0695201)**.

Coles, B.F., Anderson, K., Doerge, D.R., Churchwell, M.I., Lang, N.P. and Kadlubar, F.F., Quantitative Analysis of Inter-individual Variation of Glutathione S-Transferase Expression in Human Pancreas and the Ambiguity of Correlating Genotype with Phenotype, Cancer Research, 60(3):573-579. Accepted: 12/1/1999 **(E0699001)**.

Coles, B.F., Yang, M., Lang, N.P. and Kadlubar, F.F., Expression of GSTP1 Alleles in Human Lung and Catalytic Activity of the Native Protein Variants Towards 4-Vinylpyridine, Arachidonate Hydroperoxide, Linoleate Hydroperoxides, 1-chloro-2,4-dinitrobenzene and (+) Antibenzo[a]pyrene-7,8-diol,9-10-epoxide, Cancer Letters, 156:167-175. Accepted: 4/18/2000 **(E0689421)**.

Frame, L.T., Ozawa, S., Nowell, S.A., Chou, H., Delongchamp, R.R., Lang, N.P. and Kadlubar, F.F., A Simple Colorimetric Assay for Phenotyping the Major Human Thermostable Phenol Sulfotransferase (SULT1A1) Using Platelet Cytosols, Drug Metabolism and Disposition, 28:1063-1068. Accepted: 6/1/2000 **(NA)**.

Fu, P.P., Von Tungeln, L.S., Hammons, G.J., McMahon, G., Wogan, G., Flammang, T.J. and Kadlubar, F.F., Metabolic Activation Capacity of Neonatal Mice in Relation to the Neonatal Mouse Tumorigenicity Bioassay, Drug Metabolism Reviews, 32 (2):241-266. Accepted: 12/9/1999 **(E0687901)**.

Hammons, G.J., Yan, Y., Lopatina, N.G., Jin, B., Wise, C.K., Blann, E., Poirier, L.A., Kadlubar, F.F. and Lyn-Cook, B.A., Increased Expression of Hepatic DNA Methyltransferase in Smokers, Cell Biology and Toxicology, 15 (6):389-394. Accepted: 11/15/1999 **(E0699001)**.

Kadlubar, F.F., Hein, D., McQueen, C., Grant, D.M., Goodfellow, G.H. and Weber, W.W., Pharmacogenetics of the Arylamine *N*-Acetyltransferases: A Symposium in Honor of Wendell W. Weber, Drug Metabolism and Disposition, 28:1425-1432. Accepted: 9/11/2000 **(NA)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Lin, D., Impact of Genetic Polymorphisms in Cytochrome P450 2E1 and Glutathione S-Transferases M1, T1 and P1 on Susceptibility to Esophageal Cancer Among High-Risk Individuals in China, Cancer Epidemiology Biomarkers and Prevention, 9:551-556. Accepted: 12/30/1999 **(E0689401)**.

MacLeod, S., Nowell, S.A., Massengill, J.P., McClure, G.Y., Lang, N.P. and Kadlubar, F.F., Cancer Therapy and Polymorphisms of Cytochrome P450, Clinical Chemistry Laboratory Medicine, 38:883-887. Accepted: 1/10/2000 **(E0701501)**.

Nowell, S.A., Ambrosone, C.B., Kadlubar, F.F., Ozawa, S., Lang, N.P., MacLeod, S. and Mrackova, G., Relationship of Phenol Sulfotransferase Activity (SULT1A1) Genotype to Sulfotransferase Phenotype in Platelet Cytosol, Pharmacogenetics, 10:789-797. Accepted: 12/30/1999 **(E0300001)**.

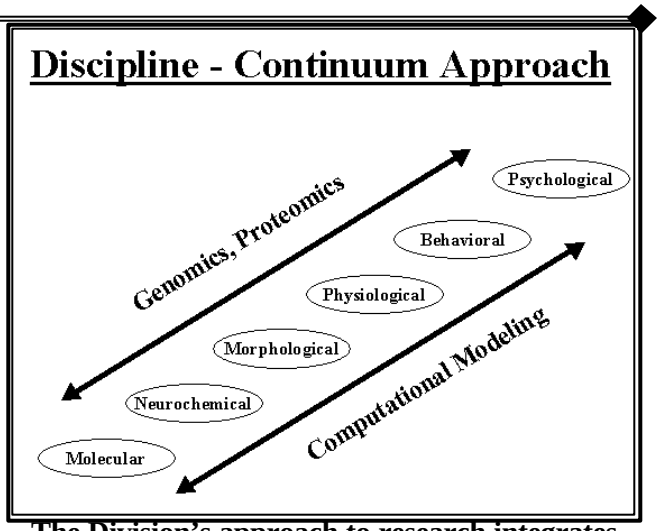
Sweeney, C., McClure, G.Y., Fares, M., Stone, A., Coles, B.F., Thompson-Carino, P., Kadlubar, F.F. and Ambrosone, C.B., Association Between Survival After Treatment for Breast Cancer and Glutathione S-Transferase P1 (GSTP1) Ile105Val Genetic Polmorphism, Journal of National Cancer Institute, 60:5621-5624. Accepted: 9/25/2000 **(E0701501)**.

Tang, Y.M., Green, B.L., Chen, G., Thompson-Carino, P., Lang, N.P., Shinde, A., Lin, D., Lyn-Cook, B.A., Hammons, G.J. and Kadlubar, F.F., Human CYP1B1 Leu 432Val Gene Polymorphism: Ethnic Distribution in African-Americans, Caucasians and Chinese: Estradiol Hydroxylase Activity; and Distribution in Prostate Cancer Cases and Controls, Pharmacogenetics, 10:761-766. Accepted: 5/17/2000 **(E0699001)**.

Yang, M., Delongchamp, R.R. and Ozawa, S., Relationship Between NAT1 Genotype and Phenotype in a Japanese Population, Pharmacogenetics, 10(3):225-232. Accepted: 2/23/2000 **(NA)**.

NEUROTOXICOLOGY

Director: ***William Slikker, Jr. Ph.D.***
Telephone: **870-543-7203**
Toll Free: **800-638-3321**
E-mail: **wslikker@nctr.fda.gov**



The Division's approach to research integrates various disciplines to solve neurotoxicological problems with the aid of genomics, proteomics and computational modeling.

Executive Summary

In the United States, brain-related disorders account for more hospitalizations than any other major disease group. One out of four Americans will suffer from a brain-related disorder at some point in their life and the estimated annual cost to the national economy for treatment, rehabilitation and related consequences is in excess of $400 billion. At no time in the past, however, have researchers been better poised to increase understanding of brain-related disorders and to reduce the risks associated with neurotoxicity.

According to a recent report from the congressional Office of Technology Assessment, "Neurotoxicity: Identifying and Controlling Poisons of the Nervous System," the known or suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides and naturally occurring substances. The number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared to those for cancer. Chemicals such as those listed above are vital to the national economy and our quality of life. The challenge is to determine at what dose and under what conditions a specific chemical may produce nervous system-related toxicity.

An interdisciplinary approach, the use of multiple, established animal models and innovative biomarkers, and an in-depth working knowledge of and experience with mechanistically-based focal areas of research enable the neurotoxicology research group to be responsive to FDA regulatory needs in a timely fashion. Several ongoing or planned studies, many in conjunction with other FDA centers, exemplify the application of the group's approach to providing critical research information applicable to FDA's regulatory problems.

Significant progress has been accomplished in the understanding of the role of body temperature and substituted amphetamine or other chemical (e.g., fenfluramine, ephedrine, MDMA, ibogaine) exposure in neuronal cell death, seizure activity, oxidative stress enzyme induction and expression of critical regulatory proteins controlling

apoptosis (e.g., bcl-2, p53 and Bax). Amelioration of neurotoxicity was observed with hypothermia or cellular energy stabilizing agents such as L-carnitine.

Developmental exposure to estrogenic agents such as genistein and nonylphenol were shown to increase salt solution intake in both sexes and decrease the volume of selected sexually dimorphic nuclei in the male rat. The time courses for neuronal cell death, astrogliosis, seizure activity and other behavioral alterations were determined in the rodent for the excitotoxicant, kainic acid, the convulsant, pilocarpine and the free radical generating organometal, aurothioglucose.

The ability to distinguish populations of normal and Attention Deficit Hyperactivity Disorder (ADHD) children and to demonstrate the 'normalizing' effects of methylphenidate was accomplished with the use of the NCTR Operant Test Battery. This same behavioral assessment tool was used to demonstrate the dose-dependent retardation of learning in developing monkeys exposed to selected anticonvulsants.

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and immediate precursor events of neurotoxicity and to use these to elucidate toxic modes of action. This will increase the certainty of assumptions underlying human risk assessments for neurotoxicants. The strategy for achieving these goals has been to develop a multidisciplinary approach integrating neuro-chemical/neurobiological (including genomics and proteomics), neuropathological, neurophysiological, and behavioral assessments to determine effects and modes of neurotoxicity. Unique features of the NCTR's neurotoxicology research efforts are the capabilities to determine target-tissue concentrations and cellular interactions of neurotoxicants and to reduce the uncertainty of extrapolating findings across species by effectively using rodent and nonhuman primate animal models as well as humans whenever possible.

Over the last decade, expertise, equipment and facilities have been woven together to pursue the overall goals of neurotoxicology research through six primary research areas. These focal areas were developed and based on prevailing scientific understanding and the importance of each area to regulatory concerns. They include mechanistically based approaches for defining and understanding the potential for a broad range of drugs and other chemicals to produce neurotoxic effects during developmental, adult or senescent life stages.

Staff will build on our strong base of dose-dependent biomarkers of effect and our unique assessment tools to focus on mechanistically based and fundamental research projects. The use of DNA array expression and proteomics tools will be further developed. Key personnel will be recruited and extensive training will be provided for existing staff so that new technologies can be incorporated into our research approach.

FY 2000 Accomplishments and FY 2001 Plans

Title	Project Number	Collaborator	Strategic Research Goal

PI: Ali, Syed

- ***Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation with Neurohistological Evaluations in Mouse and Rat Brain*** — ***E0698301*** — ***CDER*** — ***Agent-Driven***

Objective(s):

1) To determine the effects of ibogaine on dopamine, serotonin, and their metabolite concentrations in different regions of mouse and rat brain.
2) To determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain.
3) To determine the effects of ibogaine on the activities of several antioxidant enzymes: superoxide dismutase, catalase, glutathione peroxidase and glutathione levels in different regions of mouse and rat brain.
4) To evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of mouse and rat brain.
5) To determine the levels of ibogaine, noribogaine and neurohormone prolactin and corticosterone in plasma of mouse and rat.
6) To evaluate the neurohistological effects of ibogaine in different brain regions in the mouse and the rat and to correlate them with any neurochemical alterations.

FY 2000 Accomplishments:

1) All the animal treatment and tissue harvest work has been finished.
2) Tissues are in the freezer to be analyzed.
3) Four manuscripts have been published and two more have been submitted for publication.

FY 2001 Plans:

1) Analyze the data and write the manuscripts.
2) The technical report will be submitted.

- ***ADDEND: The Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation w/Neurohistological Evaluations in Mouse and Rat Brains*** — ***E0698311*** — ***CDER*** — ***Agent-Driven***

Objective(s):

To investigate if direct infusion of compounds into the brain produces similar changes in the neurotransmitter system in rats, we will inject ibogaine, noribogaine and the structurally related compound harmaline directly into the brain and will

evaluate the changes in neurotransmitter levels. Requesting additional 24 male adult Sprague Dawley rats.

FY 2000 Accomplishments:

1) Experimental part has been completed.
2) Manuscript in preparation.

FY 2001 Plans:

The technical report will be submitted.

- ***ADDEND: The Effects of Ibogaine on Neurotransmitter Systems: Correlation with Body Temperature and Electroencephalogram (EEG)*** — ***E0698321*** — ***None*** — ***Agent-Driven***

Objective(s):

To investigate what effect ibogaine might have on the electroencephalogram profile along with the time course of temperature changes in rats exposed to this compound. We would like to inject ibogaine 50 mg/kg, i.p. in five male adult Sprague-Dawley rats instrumented for the EEG and temperature recording as described in the protocol P00404.

FY 2000 Accomplishments:

1) Experimental part has been completed.
2) One manuscript has been published and one has been submitted for publication.

FY 2001 Plans:

The technical report will be submitted in early 2001.

- ***Acute Toxicity of Iron Compounds in Young Mice and Rats*** — ***E0703801*** — ***CFSAN*** — ***Agent-Driven***

Objective(s):

1) To compare acute toxicity in young animals using two forms of iron commonly used in iron supplements and one form to be used in fortification.
2) To determine if high doses of iron compounds produce reactive oxygen species, an alteration in the lipid peroxidation and changes in antioxidant enzymes in different regions of brain and liver of young mice and rats.
3) To determine the effects of high doses of iron compounds on various blood cells and the distribution of iron and iron-binding proteins in different regions of brain and other visceral organs in young animals.
4) To determine if high doses of iron compounds produce significant changes in neurotransmitter concentrations and activity of nitric oxide synthase in different regions of brain in young mice and rats.
5) To determine if high doses of iron compounds produce pathological alteration in brain and other visceral organs in young mice and rats.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

Experiments are in progress with this CFSAN collaborative project. Some data has been analyzed and is in preparation for a manuscript.

FY 2001 Plans:

Experiments will be performed according to the protocol.

◆ ***Neurotoxicity Assessment of Ephedra-containing Dietary Supplements: Application of cDNA Array and Neurochemical Approaches*** — ***X10047*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To evaluate the neurotoxicity of the eight most popular ephedra-containing dietary supplements sold in the market place and consumed by the public.
2) To determine the IC-50 of these dietary supplements using PC-12 cultured cells.
3) To determine if *in vivo* and *in vitro* exposure to these dietary supplements selectively induces specific genomic changes in PC-12 cultured cells and in different regions of mouse brain using cDNA arrays.
4) To determine if multiple doses of these dietary supplements produce significant changes in neurotransmitter concentrations in different regions of brain in mice.
5) To determine if multiple doses of these compounds produce significant changes in the formation of 3-nitrotyrosine, an *in vivo* biomarker for oxidative stress, in different regions of mouse brain.
6) To determine if multiple doses of these dietary supplements produce reactive oxygen species, alteration in the lipid peroxidation, and changes in antioxidant enzymes in different regions of mouse brain.
7) To determine if multiple doses of these dietary supplements produce pathological alteration in brain and other visceral organs in the mouse.

FY 2000 Accomplishments:

Concept approved.

FY 2001 Plans:

Develop and submit full protocol.

PI: Binienda, Zbigniew

◆ ***Metabolic Correlates of the Neurotoxicity Associated with Exposure to the Mitochondrial Inhibitor 3-nitropropionic Acid (3-NPA) in the Rat: The Role of Free Fatty Acids (FFA)*** — ***E0701001*** — ***CFSAN*** — ***Concept-Driven***

Objective(s):

1) To evaluate the acute effects of the mitochondrial inhibitor 3-NPA on brain metabolic activity using electrophysiological, neurochemical, and neurohistological endpoints:
   a) Spontaneous electrical brain activity and averaged visual evoked potentials.
   b) FFA concentration in different brain regions.
   c) Brain regional monoamine neurotransmitter concentrations: dopamine, serotonin, and their metabolites.
   d) Microscopically detectable neuronal damage.
2) To assess the possible neuroprotective effect of L-carnitine in the rat model of 3-NPA-induced histotoxic hypoxia.

FY 2000 Accomplishments:

1) A long-term experiment testing exposure to intermittent, low doses of 3-NPA has been performed, data analyzed and manuscript published.
2) *In vivo* and *in vitro* experiments have been performed to test effect of L-carnitine pre-treatment on 3-NPA -induced neurotoxicity.
3) A new assay to evaluate activity of mitochondrial succinate dehydrogenase (SDH) has been developed.
4) Histochemical assay of SDH was performed.

This project is relevant to the regulatory needs of FDA because 3-nitropropionic acid (3-NPA) is a mycotoxin that may be a contaminant of peanuts, soybeans, and cheese. It, therefore, presents a potential for human neurotoxicity. Data gathered in this protocol will provide information regarding mechanism of 3-NPA action.

FY 2001 Plans:

1) Perform a set of experiments in order to test the hypothesis of L-carnitine direct reaction with 3-NPA.
2) Continue to analyze collected data.
3) Prepare the manuscript on L-carnitine and 3-NPA.

◆ ***Experimental Autoimmune Prostatitis: Implications for the Prevention and Treatment of Inflammatory and Neoplastic Disorders of the Prostate Gland*** — ***E0701911*** — ***None*** — ***Concept-Driven***

Objective(s):

1) To induce an experimental autoimmune prostatitis in male rhesus monkeys by immunizing animals with homogenates of monkey prostate gland and mixed with Freund's adjuvant.
2) To identify the target proteins of the induced autoimmune prostatitis by using the immune sera (IgG) from the above animals to: a) screen prostate homogenates by Western immunoblot analyses; and b) screen a monkey prostate cDNA expression library.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) The immunization part of the protocol has been completed and organ homogenates obtained.
2) Several prostate-specific proteins ranging from 24 kD to 300 kD, reactive only with sera from the xenogenic (human prostate homogenate) immunization were identified.
3) Preliminary data were presented at the Arkansas Toxicology Symposium (November 1999).

PI: Bowyer, John

◆ ***Implementation of Molecular Biological Techniques for Assessing Changes in Neurogrowth/Neurotrophic Factors after Exposures to Neurotoxicants and Other Substances*** — ***E0692601*** — ***None*** — ***Method-Driven***

Objective(s):

1) To select and produce/obtain cDNA and RNA probes for detecting changes in messenger RNA (mRNA) levels for the various neurogrowth/neurotrophic factors (NTFs) which are likely to be involved in either secondary mechanisms of neurotoxicity or repair after neurotoxicant insult.
2) To detect changes in NTF mRNAs after insult to neurotoxicants and other substances, and determine if these are the same for very young and older animals.

FY 2000 Accomplishments:

The experiments to be conducted on neurotrophic factors, heat-shock/stress proteins, and catecholamine neuron-related mRNA species have been completed and published. A few of the catecholamine neuron-related mRNAs showed some potential as long-term biomarkers of amphetamine toxicity but no outstanding mRNA species were identified for this use. Also, no remarkable changes in the mRNAs of the neurotrophic species evaluated were observed.

FY 2001 Plans:

This protocol will be closed out and a final report submitted in 2001. Future endeavors to search for mRNA species suitable for biomarkers or involved in regeneration and repair of neurotoxic insults will rely much more on cDNA array analysis of mRNA. Protocol E707301 has been submitted as a vehicle for conducting such studies.

Title	Project Number	Collaborator	Strategic Research Goal
◆ ***Evaluation of the Neurotoxic Effects and Determination of the Mechanisms of Induction of Limbic Seizures Produced by Amphetamine and Related Compounds***	***E0702401***	***None***	***Concept-Driven***

Objective(s):

1) To measure the effects of dose and age on the susceptibility of amphetamine-induced limbic-type seizures in three different strains of rat and mouse, and identify areas in the brain, in particular the limbic system, where cell death and neuroplastic changes occur after amphetamine-induced seizures.
2) To determine the seizure-genic capabilities of amphetamine, phentermine, methylphenidate, and ephedrine in rat and mouse; the extracellular brain levels of these compounds necessary to induce seizures; and whether hyperthermia plays a role in the seizure induction.
3) To determine via brain microdialysis if extracellular glutamate levels are elevated in the limbic system (hippocampal rudiments and piriform cortex) prior to and during seizures induced by amphetamines.
4) To elucidate the role the noradrenergic, as well as the glutamatergic, system plays in seizures generated by amphetamines. Furthermore, begin to determine how agonists and antagonists of these two neurotransmitter systems can potentiate the seizure genesis of amphetamine.

FY 2000 Accomplishments:

Excellent progress has been made toward the goals of this protocol. Ephedrine and fenfluramine have been evaluated for their seizure-genic and neurotoxic potential in adult rats. Furthermore, some interesting findings with respect to amphetamine-induced seizures in weanling rats have led to insights into possible mechanisms of these seizures and neurotoxicity. Several papers have been published related to this work.

FY 2001 Plans:

The evaluation of ephedrine neurotoxicity in adult animals will be finished in 2001. Also, the mechanisms involved in amphetamine-induced seizures will continue to be characterized along with the use of cDNA array evaluation of mRNA species changes in regions important or involved in amphetamine-induced seizures.

◆ ***Multiple cDNA Array Analysis of the Temporal Changes in mRNA Species after Neurotoxic Events***	***E0707301***	***None***	***Predictive Toxicology***

Objective(s):

1) To develop the use of cDNA arrays as a means of detecting mRNA changes that are potential indicators of subtle and severe neurodegeneration at time points of several days up to months after neurotoxic insult.

2) To use cDNA arrays to examine changes in mRNA species that may play a role in changes in the phenotypic expression of neuronal populations in selected brain regions.
3) To expose both neuronal cell line cultures and the brain *in vivo* to neurotoxic insults and compare the changes in mRNA in the cultured cells versus specific regions of brain using cDNA arrays.
4) To compare differences in mRNA changes in specific brain regions of adult versus neonatal rats.

FY 2000 Accomplishments:

Not Applicable.

FY 2001 Plans:

Proposed start date 02/01/01.

PI: Chelonis, John

- ***Effects of Prenatal Cocaine on Behavioral Plasticity*** — ***E0663307*** — ***None*** — ***Agent-Driven***

Objective(s):

Determine whether chronic exposure to cocaine *in utero* results in long-term or residual functional consequences in rhesus monkey offspring as adults. Systematically explore how long affected subjects must be exposed to specific reinforcement contingencies before reversals of those contingencies manifest as behavioral problems.

FY 2000 Accomplishments:

Funding awarded to PI via UALR; to begin FY 2001; studies are ongoing. Presentations on findings given at International Neurochemistry Society satellite meeting, Copenhagen, Denmark, and the Annual Meeting of the Neurobehavioral Teratology Society, Palm Beach, Florida.

FY 2001 Plans:

Finalize CRADA with UALR and continue with study as defined in the grant proposal. Because numerous therapeutic agents also interact with dopaminergic systems, the information gained from such cocaine studies will have direct relevance for other 'dopaminergic' compounds of regulatory concern to the FDA.

- ***Decision Making in Children with Attention Deficit Disorder*** — ***E0703101*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine if children diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) inattentive subtype, ADHD hyperactive/impulsive subtype, and ADHD combined subtype differ from each other and children without any psychiatiric problems in their ability to delay gratification.

2) To determine if children diagnosed with ADHD inattentive subtype, ADHD hyperactive/impulsive subtype, and ADHD combined subtype, and controls differ in the degreee that they discount delayed rewards using a delay of gratification procedure in which choices are made for hypothetical amounts of money.
3) To determine for each ADHD subtype, the relationshp between severity of ADHD symptoms and delay of gratification in both of the tasks mentioned above.
4) To obtain preliminary data for determining relationships between measures of delay of gratification and other commonly used measures for assessing impulsivity in children with ADHD.

FY 2000 Accomplishments:

Preliminary data indicated a need for alteration in behavioral task parameters for this age and diagnostic group. Study on hold until changes can be effected and additional personnel (UALR students) can be recruited.

FY 2001 Plans:

If appropriate support personnel (graduate assistants and/or interns from UALR) are forthcoming, parameter changes will be explored to determine feasibility of proceeding with study.

◆ ***Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery*** ***E0703301*** ***None*** ***Agent-Driven***

Objective(s):

A battery of automated tests (games) will be given to measure aspects of learning, short-term-memory and attention, motivation, time perception, and color and position discrimination.

FY 2000 Accomplishments:

1) Study is ongoing.
2) One manuscript has been submitted, one is in press and one has been accepted.

FY 2001 Plans:

Continue data collection and workup and submit three additional manuscripts for publication.

◆ ***Complex Brain Function in Autistic Children*** ***E0704401*** ***None*** ***Agent-Driven***

Objective(s):

To compare brain functioning among autistic children and normal functioning children using tests that assess motivation, color/position discrimination, and memory. Additionally, these measures will be compared among autistic children with various degrees of symptom severity.

FY 2000 Accomplishments:

Research assistant working on project departed; therefore, the work is on hold.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Restart project if additional support personnel (interns or research assistants from UALR) can be found.

◆ ***Development of Interspecies Cognitive Assessment (Grant proposal to NIEHS submitted by PI through UALR)*** — ***X10046*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To develop operant tasks for animals that model specific cognitive functions in humans.
2) To determine whether both the clinical neuropsychological and the operant tasks are similarly sensitive to a toxicological insult by correlating lead exposures in children with performance on both types of tasks.
3) To demonstrate that monkeys and rats can perform these same operant tasks that are being used with children in this project.

FY 2000 Accomplishments:

Grant for support of work submitted to NIH.

FY 2001 Plans:

Revise grant and resubmit to NIH in June 2001.

PI: Ferguson, Sherry

◆ ***ADDEND: A Pilot Study to Assess the Effect of Developmental Genistein Exposure on Sexually Dimorphic Behaviors*** — ***E0212213*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine whether pre/neonatal exposure to genistein, a compound with estrogenic properties, will alter imprinting of sex differences in behavior.

FY 2000 Accomplishments:

Manuscript published describing the behavioral alterations resulting from developmental and chronic dietary genistein exposure. This manuscript was the first published from the NTP-supported endocrine disruptors studies here at NCTR and provides baseline data for future genistein studies as well as a basis for the current multi-generational studies.

FY 2001 Plans:

1) Data to be provided for preparation of the technical report.
2) No animals currently assigned to this protocol and no new animals to be assigned.
3) Protocol will be closed out after submission of the technical report.

Title	Project Number	Collaborator	Strategic Research Goal

- ***ADDEND: A Pilot Study to Identify Cost-Effective Sexually Dimorphic Behaviors Sensitive to the Effects of Development Exposure to Estrogenic Compounds (Methoxychlor)*** — ***E0212313*** — ***None*** — ***Agent-Driven***

  Objective(s):

  To identify easily-automated, cost-effective behavioral assays which are sexually dimorphic and sensitive to developmental exposure to environmental estrogens.

  FY 2000 Accomplishments:

  Data analyses were completed and the manuscript is in preparation.

  FY 2001 Plans:

  1) The manuscript will be submitted and all data to be provided for preparation of the technical report.
  2) No animals currently assigned to this protocol and no new animals to be assigned.
  3) Protocol will be closed out after submission of the technical report.

- ***ADDEND: A Pilot Study to Assess the Effect of Developmental Nonylphenol Exposure on Sexually Dimorphic Behaviors*** — ***E0212513*** — ***None*** — ***Agent-Driven***

  Objective(s):

  To determine whether pre/neonatal exposure to nonylphenol, a compound with estrogenic properties, will alter sex differences in behavior.

  FY 2000 Accomplishments:

  Manuscript published describing the behavioral alterations resulting from developmental and chronic dietary nonylphenol exposure. This manuscript was the second published from the NTP-supported endocrine disruptors studies here at NCTR and provides baseline data for future nonylphenol studies as well as a basis for the current multi-generational studies.

  FY 2001 Plans:

  1) Data to be provided for preparation of the technical report.
  2) No animals currently assigned to this protocol and no new animals to be assigned.
  3) Protocol will be closed out after submission of the technical report.

- ***ADDEND: A Pilot Study to Assess the Effect of Developmental Vinclozolin Exposure on Sexually Dimorphic Behavior*** — ***E0212613*** — ***None*** — ***Agent-Driven***

  Objective(s):

  To determine whether pre/neonatal exposure to vinclozolin, a compound with potential estrogenic properties, will alter sex differences in behavior.

FY 2000 Accomplishments:

Manuscript in press describing the behavioral alterations resulting from developmental and chronic dietary vinclozolin exposure. This manuscript provides baseline data for future vinclozolin studies as well as a basis for risk assessment.

FY 2001 Plans:

1) Data to be provided for preparation of the technical report.
2) No animals currently assigned to this protocol and no new animals to be assigned.
3) Protocol will be closed out after submission of the technical report.

◆ ***ADDEND: A Pilot Study to Assess the Effect of Developmental Ethinyl Estradiol Exposure on Sexually Dimorphic Behaviors*** — ***E0212913*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine whether pre/neonatal exposure to ethinyl estradiol, a compound with potential estrogenic properties, will alter sex differences in behavior.

FY 2000 Accomplishments:

Data analyses are complete. Data from the behavioral studies were used by the Toxicology Study Selection and Review Committee (TSSRC) to establish appropriate doses for the beginning multi-generational studies.

FY 2001 Plans:

1) Manuscript will be completed and submitted.
2) No animals currently assigned to this protocol and no new animals to be assigned.
3) Protocol will be closed out after submission of the technical report.

◆ ***ADDEND: The Effects of Developmental/Chronic Genistein Exposure over Multiple Generations on Maternal, Play, Mating/Reproductive Behaviors and Neurochemical Measures*** — ***E0213213*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine whether chronic exposure of rats over multiple generations to genistein, a compound with potential estrogenic properties, will alter maternal behavior, play behavior of either sex, the female lordosis response, male mating behavior, or the amphetamine-induced release of striatal dopamine, which is known to be estrogen-modulated.

FY 2000 Accomplishments:

Two manuscripts in press. One describes the effects of dietary genistein exposure on maternal behavior and the importance of examining maternal behavior in studies of endocrine disruptors with estrogenic actions. The other manuscript reviews the developmental neurotoxicity of endocrine disruptors.

FY 2001 Plans:

1) Two additional manuscripts will be completed and submitted.
2) There are no animals assigned to this protocol.

◆ ***ADDEND: The Effects of Developmental/Chronic Nonylphenol Exposure over Multiple Generations on Sexually Dimorphic Behaviors, and Neurochemical Measures*** — ***E0213513*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine whether chronic exposure of rats over multiple generations to nonylphenol, a compound with potential estrogenic and/or androgenic properties, will alter maternal behavior, the female lordosis response, male mating behavior, sodium solution intake, amphetamine-induced release of the striatal dopamine, or serum levels of testosterone and estradiol in males.

FY 2000 Accomplishments:

Data were collected on schedule and several portions of the study have been statistically analyzed.

FY 2001 Plans:

1) Data analyses will continue and data will be reported at the 2001 meeting of the Society of Toxicology.
2) There are no animals assigned to this protocol.

◆ ***Validity of Developmental Cerebellar Stunting in the Rat as a Model for Attention Deficit Hyperactivity Disorder: Behavior and Neurochemistry*** — ***E0704001*** — ***None*** — ***Concept-Driven***

Objective(s):

1) To identify treatments which cause developmental cerebellar stunting, specifically those which decrease the granule cell population with few effects on Purkinje cells.
2) To confirm the increase in locomotor activity caused by developmental cerebellar stunting and to determine the degree to which this hyperactivity resembles human ADHD.
3) To identify other behavioral alterations associated with developmental cerebellar stunting and to determine the degree to which these resemble those associated with human ADHD.
4) To identify the neurochemical alterations in different brain regions resulting from the developmental insult.
5) To compare these neurobehavioral and neurochemical alterations to those exhibited by the most common rodent model of ADHD: the Spontaneously Hypertensive Rat (SHR).

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) Two manuscripts published.
2) A study of the Spontaneously Hypertensive Rat, its normotensive control, the Wistar-Kyoto Rat and the NCTR Strain 23 (Sprague-Dawley rat) is currently in progress. Preliminary results indicate that the SHR shows severe behavioral alterations which are heretofore unreported.

FY 2001 Plans:

1) At least two additional manuscripts will be submitted describing DFMO-induced behavioral and neurochemical alterations.
2) Results from the SHR study will be presented at scientific meetings.
3) A study of dexamethasone-induced behavioral alterations will begin in the early spring.

PI: Flynn, Katherine

◆ ***ADDEND: The Effects of Nonylphenol Exposure over Multiple Generations on Cognitive Functions and Hippocampal Structure in Female Rats*** — ***E0213521*** — ***None*** — ***Agent-Driven***

Objective(s):

To determine whether chronic exposure over multiple generations to nonylphenol, a compound with estrogenic properties, will alter performance on learning/memory tasks and/or hippocampal structure in young adult and middle-aged female rats.

FY 2000 Accomplishments:

A manuscript has been submitted.

FY 2001 Plans:

1) There are no animals assigned to this protocol.
2) Protocol will be closed out after submission of the technical report.

PI: Patterson, Tucker

◆ ***Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys*** — ***E0250201 E0250211*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-dideoxycytidine (ddC) and thalidomide in rhesus monkeys.

FY 2000 Accomplishments:

Behavioral data analyzed.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Prepare manuscript for submission.

PI: Paule, Merle

- ***Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions (97032)- ASTRA CRADA*** — ***E0280001*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To establish acquisition curves for several operant behaviors in juvenile rhesus monkeys during chronic oral exposure to two anticonvulsant agents and vehicle.
2) To determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behavior.
3) To determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters.
4) To determine plasma distribution profiles and concentrations for each of these agents at various stages of chronic exposure.

FY 2000 Accomplishments:

1) In-life portion of study complete.
2) CRADA report supplied to NCTR Quality Assurance (QA) and sponsor.
3) Four presentations of study findings were made:
   a) Two at the Annual Meeting of the Neurobehavioral Teratology Society, Palm Springs, Florida.
   b) One at the 5th International Conference on Neuroprotective Agents, Lake Tahoe, California.
   c) One at the 18th International Neurotoxicology Conference, Colorado Springs, Colorado.

FY 2001 Plans:

1) Final report filed.
2) Two manuscripts submitted for publication, one more to be submitted.

- ***ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions*** — ***E0280011*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To determine whether the effects of chronic remacemide treatment are due to reversible effects linked to daily drug exposure or are due to irreversible central nervous system (CNS) toxicity.
2) To propose to monitor behavioral acquisition in subjects during six months of reduced drug exposure.

3) To request extension of project requiring additional housing/maintenance and research time.

FY 2000 Accomplishments:

1) In-life portion of study complete.
2) CRADA report supplied to NCTR QA and sponsor.
3) Four presentations of study findings were made:
   a) Two at the Annual Meeting of the Neurobehavioral Teratology Society, Palm Springs, Florida.
   b) One at the 5th International Conference on Neuroprotective Agents, Lake Tahoe, California.
   c) One at the 18th International Neurotoxicology Conference, Colorado Springs, Colorado.

FY 2001 Plans:

1) Final report filed.
2) Two manuscripts submitted for publication, one more to be submitted.

◆ ***ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions*** — *E0280021* — *None* — *Predictive Toxicology*

Objective(s):

To continue study by requesting pathology support for sacrifice of 29 nonhuman primates on this study; also requesting extension of project for two months in order to monitor subjects' behavioral acquisition for one month during which subjects are not subject to any dosing procedures.

FY 2000 Accomplishments:

1) In-life portion of study complete.
2) CRADA report supplied to NCTR QA and sponsor.
3) Four presentations of study findings were made: two at the Annual Meeting of the Neurobehavioral Teratology Society, Palm Springs, Florida; one at the 5th International Conference on Neuroprotective Agents, Lake Tahoe, California; and one at the 18th International Neurotoxicology Conference, Colorado Springs, Colorado.

FY 2001 Plans:

1) Final report filed.
2) Two manuscripts submitted for publication, one more to be submitted.

◆ ***Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions/Rodent equivalent.*** — *E0280031* — *None* — *Predictive Toxicology*

Objective(s):

To run rodent studies to parallel those completed in the nonhuman primate under E0280001**.**

FY 2000 Accomplishments:

1) Protocol proposal submitted.
2) Concurrence in support of project by other members of FDA (CDER) sought.

FY 2001 Plans:

1) Protocol submitted, in collaboration with previous sponsor.
2) CRADA extended to aid in support of this rodent version of the study.

◆ ***ADDEND: Preliminary Studies for Determining the Effects of Chronic Cocaine Exposure during Pregnancy on the Behavior of Offspring in Monkeys*** ***E0663306*** ***None*** ***Agent-Driven***

Objective(s):

To increase the number of offspring in the total gestational exposure (TGE) group to ten. Requesting that ten nonpregnant animals be maintained under chronic cocaine treatment while they are in the breeding program until at least ten viable offspring are available. Requesting another seven animals for inclusion in control group to bring the total to ten.

FY 2000 Accomplishments:

1) Partial support of this work was obtained via a B-START UALR grant.
2) This research will be extended under E0663307.
3) Three research presentations were made.

FY 2001 Plans:

Protocol rolled over into E663307, funding via CRADA with UALR.

◆ ***Effects of Chronic Methylphenidate (Ritalin) Administration on 'cognitive' Functions in the Rhesus Monkey*** ***E0683700*** ***None*** ***Agent-Driven***

Objective(s):

To determine whether chronic treatment with relevant doses of the therapeutic agent methylphenidate (Ritalin) will result in detectable changes in specific 'cognitive' abilities in a nonhuman primate model of complex brain function.

FY 2000 Accomplishments:

1) In-life portion of study completed; awaiting behavioral data analysis to allow proceeding with publications.
2) Preliminary report on pathology provided to NCTR administration, awaiting decision on when and how to proceed with pathology data.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

1) Await behavioral data analysis for interpretation and write-up for publication.
2) Await consultation with administration concerning disposition of pathology data.

◆ ***Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity*** — ***E0691401*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To determine the acute effects of a variety of prototypic psychotropic agents on rodent performance in an Operant Test Battery (OTB) containing tasks designed to model several complex brain functions.
2) To determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption.
3) To compare and contrast the acute effects of these psychotropic agents on rodent and primate OTB performance to determine the degree to which behavioral findings in rodents can be extrapolated to primates.
4) To validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity.
5) To add to existing knowledge of the neurochemical and neurophysiological basis of complex brain functions.

FY 2000 Accomplishments:

1) In-life data collection stopped.
2) Prepared data analysis requests.
3) One scientific presentation was made at the Annual Meeting of the Society for Neuroscience and three were made at the 17th International Neurotoxicology Conference.
4) Five publications in preparation for submission.

FY 2001 Plans:

1) Continue data interpretation and analysis.
2) Prepare manuscripts for publication and completion of final report.

◆ ***ADDEND: Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity*** — ***E0691411*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To transfer 24 rats from P00327 to E0691401 and add fenfluramine to the list of agents to be evaluated under E0691401. Transfer of animals will enable researcher to evaluate the acute effects of the various psychotropic agents outlined in E0691401.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2000 Accomplishments:

1) In-life data collection stopped.
2) Prepared data analysis requests.
3) One scientific presentation was made at the Annual Meeting of the Society for Neuroscience and three were made at the 17th International Neurotoxicology Conference.

FY 2001 Plans:

1) Continue data interpretation and analysis.
2) Prepare manuscripts for publication.
3) Complete the final report.

◆ ***ADDEND: Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity*** — ***E0691421*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To administer one additional known or putative neurotoxicant to rats and then to monitor their behavior for approximately one additional month.
2) To require additional dosing requirements.

FY 2000 Accomplishments:

1) In-life data collection stopped.
2) Prepared data analysis requests.
3) One scientific presentation was made at the Annual Meeting of the Society for Neuroscience and three were made at the 17th International Neurotoxicology Conference.

FY 2001 Plans:

1) Continue data interpretation and analysis.
2) Prepare manuscripts for publication.
3) Complete final report.

◆ ***Use of the NCTR Nonhuman Primate Operant Test Battery (OTB) as a Predictor of Acute Neurobehavioral Toxicity: Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes*** — ***E0697901*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To further explore the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems.

2) To determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance.
3) To characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB.
4) To more thoroughly characterize the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems.
5) To determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.

FY 2000 Accomplishments:

1) In-life data collection ended.
2) Data analysis and interpretation.
3) Two publications prepared for submission.

FY 2001 Plans:

1) Continue data interpretation pending completion of requested analyses.
2) Continue to publish findings and compile final report.

◆ ***Risk Factors for Attention-Deficit/Hyperactivity Disorder (ADHD)*** — ***X60043*** — ***None*** — ***Concept-Driven***

Objective(s):

This project will involve an epidemiologic study of several possible environmental risk factors associated with the occurrence of ADHD in a large population of school-age children (grades 1-5). Components of the NCTR Operant Test Battery will be used to determine whether performance of these tasks is associated with the clinical diagnosis of ADHD status.

FY 2000 Accomplishments:

No activity.

FY 2001 Plans:

Keep protocol open pending support from NIEHS collaborators.

PI: Scallet, Andrew

◆ ***ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: II. Genistein*** — ***E0212215*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine whether developmental exposure to genistein may modify the sexually dimorphic areas of the adult rodent brain.

2) To compare neurochemical and neurohistological biomarkers of genistein exposure for their relative sensitivity and concordance.

FY 2000 Accomplishments:

1) Genistein was identified as one of a family of estrogenic compounds that interfered with normal sexual differentiation by feminizing the development of the genetically male rat brain.
2) A mathematical dose-response model for comparing and predicting the effects of genistein with those of other estrogenic compounds was developed. These models should have predictive value for determining the risk for this type of neurotoxic outcome as a result of exposure to various other estrogenic compounds.

FY 2001 Plans:

1) Submitted a description of findings on genistein for publication.
2) Prepare the paper on dose-response modeling for publication.

◆ ***ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: I. Methoxychlor*** — ***E0212315*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine whether developmental exposure to methoxychlor may modify the sexually dimorphic areas of the adult rodent brain.
2) To compare neurochemical and neurohistological biomarkers of methoxychlor exposure for their relative sensitivity and concordance.

FY 2000 Accomplishments:

Relative dose-exposure parameters for methoxychlor were determined, as well as estimates of its effects on the sexual differentiation of the rat brain. No effects on sexual differentiation were observed, perhaps because of the relatively low amounts of effective estrogenic activity that were achieved by the dosing regimen.

FY 2001 Plans:

A final report based on these findings will be prepared and submitted to the study sponsors (NTP).

◆ ***ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: III. Nonylphenol*** — ***E0212515*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine whether developmental exposure to nonylphenol may modify the sexually dimorphic areas of the adult rodent brain.

2) To compare neurochemical and neurohistological biomarkers of nonylphenol exposure for their relative sensitivity and concordance.

FY 2000 Accomplishments:

1) Nonylphenol was identified as one of a family of estrogenic compounds that interfered with normal sexual differentiation by feminizing the development of the genetically male rat brain.
2) A mathematical dose-response model for comparing and predicting the effects of nonylphenol with those of other estrogenic compounds was developed. These models should have predictive value for determining the risk for this type of neurotoxic outcome as a result of exposure to various other estrogenic compounds.

FY 2001 Plans:

1) Submit a description of our findings on nonylphenol for publication.
2) Prepare the paper on dose-response modeling for publication.

◆ ***ADDEND: Neurotoxicological Effects of Exposure to an Anti-Androgenic Compound during Development: Vinclozolin*** — ***E0212615*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine whether developmental exposure to vinclozolin may modify the sexually dimorphic areas of the adult rodent brain.
2) To compare neurochemical and neurohistological biomarkers of vinclozolin exposure for their relative sensitivity and concordance.

FY 2000 Accomplishments:

1) Examined the potential effects of vinclozolin on the sexual differentiation of the male and female rat hypothalamus (central nucleus of the medial preoptic region). No alterations were found in the structure of this brain region.
2) A manuscript describing the lack of effects of vinclozolin in relation to the interference with normal sexual differentiation caused by estrogenic compounds is being prepared for publication.

FY 2001 Plans:

Submit for publication a manuscript describing the lack of effects of vinclozolin on sexual differentiation of the central nucleus of the preoptic region of the hypothalamus.

◆ ***ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: V. Ethinyl Estradiol*** — ***E0212915*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To determine whether developmental exposure to ethinyl estradiol may modify the sexually dimorphic areas of the adult rodent brain.

2) To compare neurochemical and neurohistological biomarkers of ethinyl estradiol exposure for their relative sensitivity and concordance.

FY 2000 Accomplishments:

1) Ethinyl estradiol was identified as one of a family of estrogenic compounds that interfered with normal sexual differentiation by feminizing the development of the genetically male rat brain.
2) A mathematical dose-response model for comparing and predicting the effects of ethinyl estradiol with those of other estrogenic compounds was developed. These models should have predictive value for determining the risk for this type of neurotoxic outcome as a result of exposure to various other estrogenic compounds.

FY 2001 Plans:

1) Submit a description of our findings on ethinyl estradiol for publication.
2) Prepare the paper on dose-response modeling for publication.

◆ ***ADDEND: Multigenerational Exposure to Estrogenic Compounds: I. Genistein Effects on Volume of the Sexually Dimorphic Nucleus*** — ***E0213215*** — ***None*** — ***Agent-Driven***

Objective(s):

To evaluate the hypothesis that multigenerational exposure to genistein may produce a reduction in the volume of the male sexually dimorphic nucleus of the medial preoptic area of the hypothalamus.

FY 2000 Accomplishments:

1) Obtained, dissected, processed, sectioned, and stained approximately one-fourth of the male brains sacrificed for pathology from the parent protocol on multigenerational effects of genistein.
2) Trained a new full-time support scientist from Pathology to assist with these studies, as well as a half-time individual to operate the Image Analysis equipment.

FY 2001 Plans:

Complete the Image Analysis of the male brains in order to see if this second experiment on genistein also shows that it can feminize the sexual differentiation of genetically male rats, as was observed in a previous experiment.

◆ ***ADDEND: Multigenerational Exposure to Estrogenic Compounds: II. Nonylphenol Effects on Volume of the Sexually Dimorphic Nucleus*** — ***E0213515*** — ***None*** — ***Agent-Driven***

Objective(s):

To evaluate the hypothesis that multigenerational exposure to nonylphenol may produce a reduction in the volume of the male sexually dimorphic nucleus of the medial preoptic area of the hypothalamus.

FY 2000 Accomplishments:

None.

FY 2001 Plans:

Complete the dissection, processing, sectioning, staining and measurement of the brains, through the stages of computer-image analysis and data presentation and evaluation.

- ***Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure*** — ***E0693001*** — ***CFSAN*** — ***Agent-Driven***

Objective(s):

1) To correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated quantitative neurohistological and behavioral effects in non-human primates.
2) To identify genetic factors modulating domoic acid sensitivity in Wistar rats.
3) To identify neurochemical biomarkers of domoic acid exposure and damage.

FY 2000 Accomplishments:

Domoic acid is a potent neurotoxin formed by algal blooms that enters the human food chain when various types of seafood are consumed. Further dose-response data evaluating the initial location of the early-immediate gene (c-fos) response to domoic acid exposure was obtained. This serves to better identify the initial target tissue for domoic acid's neurotoxicity and will subsequently allow for more precise determination of safe levels of exposure. In turn, safety experts will have better information on which to base economic decisions such as when commercial crabbing and fishing operations must be suspended for public safety.

FY 2001 Plans:

Two additional manuscripts describing the dose-related neurotoxicity of domoic acid are planned for submission in FY 2001.

- ***Perinatal Biomarkers of Exposure in Genistein: Effects on Testosterone and Neuronal Estrogen Receptor Ligation during Development*** — ***X00050*** — ***None*** — ***Agent-Driven***

Objective(s):

1) To develop a dosing regimen with genistein and measure the resulting blood levels of genistein and testosterone in perinatal-aged male rats.
2) To locate estrogen receptors and aromatase, within the brains of perinatal-aged rodents exposed to genistein.
3) To evaluate the rate of neuronal apoptosis and proliferation in the sexually dimorphic hypothalmic nuclei (SDN) of perinatal-aged rats exposed to genistein.

4) To explain the bimodal dose-response curve previously observed for chronic dietary exposure to genistein and other endocrine disruptors.
5) To expand dose-response comparisons for the three primary endpoints to other endocrine disrupting compounds; nonylphenol and ethinyl estradiol.

FY 2000 Accomplishments:

The protocol has been extended to add a consideration of the effects of very small amounts of estrogenic compounds on both testosterone levels as well as the binding of these agents to estrogen receptors located in the developing hypothalamus. The additional biomarkers should comprise a very useful screening system to identify compounds that may provide similar hazards for the normal sexual differentiation of the brain.

FY 2001 Plans:

If approved, this protocol will be initiated in FY 2001.

PI: Schmued, Laurence

◆ ***Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants.***

Project Number	Collaborator	Strategic Research Goal
E0701301 *E0701311* *E0701321* *E0701331*	*None*	*Predictive Toxicology*

Objective(s):

1) To develop and validate a battery of conventional and novel histochemical techniques for resolving the nature, distribution and underlying mechanisms of brain damage resulting from exposure to FDA-relevant neurotoxicants.
2) To localize throughout the central nervous system, histochemical and pathological changes resulting from exposure to different classes of neurotoxicants.
3) To develop the ability to predict the neuroanatomical regions at risk and the potential functional consequences of exposure to the neurotoxicant of interest, by correlating a compound's putative mode of action with a characteristic histochemical profile.

FY 2000 Accomplishments:

I. Methodological Accomplishments

- Developed Black-Gold, a histochemical tracer for the simple high resolution staining of normal and pathological myelin. This is relevant to the FDA, in that it directly benefits both its own researchers as well as industrial veterinary pathologists in that it provides them with a new tool for better assessing toxicant-induced myelin changes.
- Developed Fluoro-Jade B, a novel histochemical tracer with the highest affinity for degenerating neurons. This is relevant to the FDA in that it directly benefits both its own researchers as well as industrial veterinary pathologists because it provides them with a new tool for better localizing toxicant-induced neuronal degeneration.

II. Applied and Conceptual Accomplishments

- Characterized the temporal neuronal and glial changes associated with excitotoxic agents such as kainic acid and domoic acid. These compounds are of relevance to the FDA since the former has been used as an ascaricide and the latter has been found in shellfish, potentially resulting in irreversible brain damage.
- Characterized the neuronal and glial pathologies following exposure to free-radical-generating organometal compounds such as aurothioglucose. This compound is of relevance to the FDA since it is widely used to treat advanced rheumatoid arthritis.
- Characterization in progress of the neuronal and glial pathologies following exposure to cholinergic agonists such as pilocarpine. Pilocarpine is of relevance to the FDA since it is prescribed for glaucoma, xerostomia (a side effect of radiation therapy), and Sjogren's syndrome (an autoimmune disease).
- Characterization in progress of the neuronal degeneration resulting from acute exposure to methyldioxymethamphetamine (MDMA, Ecstasy). This is of relevance to the FDA since MDMA is the fastest-growing drug of abuse in this country, and as an aromatic monoamine agonist, it shares many properties with pharmaceutical antidepressants and anorexics.

FY 2001 Plans:

I. Continuation of Histochemical Test Battery:

A. Additional Methods Development:

- Develop a vital histochemical tracer that will result in both functional activation of a brain region, while also revealing the underlying neuronal connectivity.
- As Fluoro-Jade B contains two to four related compounds, we hope to isolate and determine the biological activity and chemical structure of each component.

B. Additional Classes of Neurotoxicants to be Characterized by Pathology:

- Inhibitors of oxidative metabolic respiration. It should be informative to look at compounds that act to inhibit different specific mitochondrial complexes such as MPTP or rotenone (Complex I), 3-NPA (Complex II), actinomycin A (Complex III), and cyanide or azide (Complex IV).
- Complete characterization of neuronal degeneration resulting from exposure to aromatic monoamine agonists including MDMA.

II. New protocols to be proposed:

A. Resolve the potential neurotoxicity of three putative Alzheimer therapeutic agents (phencyclidine, prednesone and trisodium dicarboxyfluorescein in APP transgenic mice. This should be relevant to the FDA since an unprecedented number of Alzheimer's Disease therapeutics are presently under development.

B. Characterize the protein expressed only in degenerating neurons. It is believed that such a study would be significant as it could provide insights on

both the chemistry and underlying mechanism of toxicant-induced neuronal degeneration. It also may be relevant by allowing identification of a pathological protein that could be targeted by therapeutic agents, much the way drugs are designed to prevent or dissolve amyloid plaques in Alzheimer's Disease.

PI: Slikker, William

- ***Quantitative Procedures for Neurotoxicity Risk Assessment*** — ***E0310001*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To determine the necessary parameters for a biologically based dose-response model to predict neurotoxic adverse effects following exposure to cholinesterase-inhibiting pesticides. Such information would improve the ability of risk assessments to evaluate toxicological data for potential human health risk and address a specific need identified by the Neurotoxicity Risk Assessment Guidelines.

FY 2000 Accomplishments:

This EPA/NCTR IAG was established, postdoctoral support recruited and hired, and essential equipment purchased.

FY 2001 Plans:

Develop a preliminary biologically based dose-response model for foodborne pesticides.

- ***Validation Study of the Physiologically Based Pharmacokinetic (PBPK) Model for Description of Low-dose, Long-term Exposure of 2,4-dichlorophenoxyacetic Acid (2,4-D) Dosimetry in the Central Nervous System (CNS)*** — ***E0699201*** — ***CFSAN*** — ***Predictive Toxicology***

Objective(s):

To obtain CNS pharmacokinetic profiles of 2,4-D transport in the rat after low-dose, chronic dosing (28 days). The data will be used to validate the previously developed PBPK model which simulates the uptake, distribution, and clearance of 2,4-D.

FY 2000 Accomplishments:

First manuscript describing the model development was written and submitted.

FY 2001 Plans:

Second manuscript and final report will be submitted. This CFSAN collaboration project served as a partial basis for the EPA/NCTR/FDA IAG.

Title	Project Number	Collaborator	Strategic Research Goal

- ***Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey*** — ***E0702601 E0702611 E0702621*** — ***CDER*** — ***Predictive Toxicology***

Objective(s):

1) To determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine.
2) To determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine.

FY 2000 Accomplishments:

1) In-life phase of study completed.
2) Ultrasound preliminary pathology and clinical chemistries completed.

FY 2001 Plans:

Complete pharmacokinetic and behavioral data analysis and submit final report.

- ***Disposition and Effect of Thimerosal (Ethyl Mercury) in the Developing Mammal*** — ***X00016*** — ***None*** — ***Predictive Toxicology***

Objective(s):

1) To determine the applicability of methyl mercury exposure guidelines to Thimerosal (ethyl mercury) exposure from vaccines by comparing toxicokinetic profiles of ethyl mercury vs. methyl mercury in the monkey.
2) To assess the developmental neurotoxicity potential of Thimerosal in the developing monkey.

FY 2000 Accomplishments:

Collaborative connections established with CBER/CDER for preliminary proposal developed.

FY 2001 Plans:

Develop full Thimerosal proposal in multiple animal species including nonhuman primate and establish funding mechanism via NTP or other source.

- ***A Rhesus Monkey Model for Production of Fetal Hypoxic Ischemic Brain Injury*** — ***X00047*** — ***None*** — ***Predictive Toxicology***

Objective(s):

To develop an aortic surgical technique for use in the term rhesus monkey that will induce hypoxic-ischemic injury to the fetal brain with anatomic and pathologic distribution similar to those seen in the human newborn.

FY 2000 Accomplishments:

1) Research team at University of Arkansas for Medical Sciences, Arkansas Children's Hospital, and NCTR formed (imaging capability).
2) Initial grant proposal drafted.

Title	Project Number	Collaborator	Strategic Research Goal

FY 2001 Plans:

Submission of grant proposal on perinatal hypoxia.

PI: Xu, Zengjun

◆ ***Study on Alteration of the Programming of the 5-HT System by Nicotine Exposure during Development*** | ***X10054*** | ***None*** | ***Predictive Toxicology***

Objective(s):

1) To determine how prenatal nicotine exposure alters 5HT synaptic function known to be targeted in models of 5HT dysfunction.
2) Evaluate development of 5HT signal transduction assessed with receptor ligand binding, linkages to adenyl cyclase and DNA expression arrays.
3) To determine whether the critical period for nicotine-induced alterations in the programming of 5HT function extends into adolescence.
4) Assess 5HT synaptic function during adolescent nicotine treatment and withdrawal, using the same endpoints as studies with the prenatal nicotine mode.

FY 2000 Accomplishments:

Protocol concept submitted.

FY 2001 Plans:

The protocol concept approved; therefore, a full protocol will be submitted.

FY 2000 Publications

Baumann, M.H., Rothman, R.B. and Ali, S.F., Is Ibogaine Like Mk-801? A Comparative Neurobiological Investigation, Neuropharmacology, 59:143-151. Accepted: 1/21/2000 **(E0698301)**.

Binienda, Z.K., Scallet, A.C., Schmued, L.C. and Ali, S.F., Ibogaine Neurotoxicity Assessment: Electrophysiological, Neurochemical, and Neurohistological Methods, The Alkaloids (Academic Press). Accepted: 5/15/2000 **(E0698321)**.

Bondy, S.C., Guo-Ross, S. and Ali, S.F., Aluminum but Not Iron Treatment Induces Pro-Oxidant Events in the Rat Brain, Brain Research, 34(2-3):219-232. Accepted: 1/24/2000 **(E0703801)**.

Bowyer, J.F., Neuronal Degeneration in the Limbic System of Weanling Rats Exposed to Saline, Hyperthermia or D-Amphetamine, Brain Research. Accepted: 8/29/2000 **(E0702401)**.

Bowyer, J.F., Newport, G.D., Slikker, W., Gough, B.J., Ferguson, S.A. and Tor-Agbidye, J., An Evaluation of L-Ephedrine Neurotoxicity With Respect to Hyperthermia and Caudate/Putamen Microdialysate Levels of Ephedrine Dopamine, Serotonin and Glutamate, Toxicological Sciences, 55 (1):133-142. Accepted: 12/22/1999 **(E0702401)**.

Cada, A.M., De La Torre, J.C. and Gonzalez-Lima, F., Chronic Cerebrovascular Ischemia in Aged Rats: Effects on Brain Metabolic Capacity and Behavior, Neurobiology of Aging, 225-233. Accepted: 1/31/2000 **(NA)**.

Cada, A.M., Gray, E.P. and Ferguson, S.A., Minimal Behavioral Effects From Developmental Cerebellar Stunting in Young Rats Induced by Postnatal Treatment With α-Difluoromethylornithine, Neurotoxicology and Teratology, 22 (3):415-420. Accepted: 12/7/1999 **(E0704001)**.

Chang, H.C., Rountree, R.L. and Leu, L., Electron Microscopic Observation of Unripe Banana Fruit Infected With *Colletotrichum musae*, Plant Protection Bulletin, 42:135-146. Accepted: 5/24/2000 **(NA)**.

Chelonis, J.J., Edwards, M.C., Schulz, E.G., Baldwin, R., Wenger, A. and Paule, M.G., Methylphenidate Normalizes Recognition Memory in Children Diagnosed With Attention Deficit/Hyperactivity Disorder, Journal of Experimental and Clinical Psychopharmacology. Accepted: 7/6/2000 **(E0703101)**.

Doerge, D.R., Fogle, C.M., Paule, M.G., McCullagh, M. and Bajic, S., Analysis of Methylphenidate and Its Metabolite Ritalinic Acid in Monkey Plasma by Liquid Chromatography/Electrospray Ionization Mass Spectrometry, Rapid Communications in Mass Spectrometry, 14:619-623. Accepted: 2/4/2000 **(E0692001)**.

Ferguson, S.A., Flynn, K.M., Delclos, K.B. and Newbold, R., Maternal and Offspring Toxicity but Few Sexually Dimorphic Behavioral Alterations Result From Nonylphenol Exposure, Neurotoxicology and Teratology, 22:583-591. Accepted: 1/14/2000 **(E0212513)**.

Ferguson, S.A., Frisby, N.B. and Ali, S.F., Acute Effects of Cocaine on Play Behavior of Rats, Behavioral Pharmacology, 11:175-179. Accepted: 12/16/1999 **(E0684600)**.

Ferguson, S.A., Scallet, A.C., Flynn, K.M., Meredith, J.M. and Schwetz, B.A., Developmental Neurotoxicity of Endocrine Disruptors: Focus on Estrogens, Neurotoxicology. Accepted: 7/18/2000 **(E0212201)**.

Flynn, K.M., Ferguson, S.A., Delclos, K.B. and Newbold, R., Effects of Genistein Exposure on Sexually Dimorphic Behaviors, Toxicological Sciences, 55 (2):311-319. Accepted: 2/4/2000 **(E0212213)**.

Flynn, K.M., Ferguson, S.A., Delclos, K.B. and Newbold, R., Multigenerational Exposure to Genistein Has Minimal Effects on Maternal Behavior in Rats, NeuroToxicology. Accepted: 7/5/2000 **(E0213213)**.

Hashemi, R.R., Tyler, A.A., Epperson, C. and Young, J.F., Knowledge Discovery From Sparse Pharmacokinetic Data, Proceedings of SAC 2000 - 15th ACM Symposium in Applied Computing. Accepted: 1/20/2000 **(P00393)**.

Holson, R.R., Adams, J., Ferguson, S.A. and Scalzo, F.M., Retinoic Acid Exposure on Gestational Days 11-13 Impairs Swallowing in Rat Offspring, Neurotoxicology and Teratology, 22:541-545. Accepted: 5/1/2000 **(E0690501)**.

Hopkins, K.J., Wang, G.J. and Schmued, L.C., Temporal Progression of Kainic Acid-Induced Neuronal and Myelin Degeneration in the Rat Forebrain, Brain Research, 864:69-80. Accepted: 2/7/2000 **(E0701301)**.

Imam, S.Z. and Ali, S.F., Selenium, An Antioxidant, Attenuates Methamphetamine-Induced Dopaminergic Toxicity and Peroxynitrite Generation, Brain Research, 855:186-191. Accepted: 10/25/1999 **(E0703801)**.

Imam, S.Z., Islam, F., Itzhak, Y., Slikker, W. and Ali, S.F., Prevention of Dopaminergic Neurotoxicity by Targeting Nitric Oxide and Peroxynitrite: Implications for the Prevention of Methamphetamine-Induced Neurotoxic Damage, New York Academy of Sciences, 914:157-171. Accepted: 7/19/2000 **(NA)**.

Johnson, J.R., Robinson, B.L., Ali, S.F. and Binienda, Z.K., Dopamine Toxicity Following Long-Term Exposure to Low Doses of 3-Nitropropionic Acid in Rats, Toxicology Letters, 116:113-118. Accepted: 4/26/2000 **(E0701001)**.

Kwon, O., Slikker, W. and Davies, D.L., Biochemical and Morphological Effects of Fumonisin B_1 on Primary Cultures of Rat Cerebrum, Neurotoxicology and Teratology, 22:565-572. Accepted: 4/5/2000 **(E0688401)**.

Lu, M., Tang, N. and Ali, S.F., Effect of Single Injection of Methylazoxymethanol at Postnatal Day One on Cell Proliferation in Different Brain Regions of Male Rats, NeuroToxicology. Accepted: 6/19/2000 **(P00406)**.

Mayorga, A.J., Fogle, C.M. and Paule, M.G., Similar Effects of Amphetamine and Methylphendidate on the Performance of Complex Operant Tasks in Rats, Behavioural Brain Research, 109:59-68. Accepted: 11/5/1999 **(E0691401)**.

Meredith, J.M., Bennett, C.A. and Scallet, A.C., A Practical Three-Dimensional Reconstruction Method to Measure the Volume of the Sexually-Dimorphic Central Nucleus of the Medial Preoptic Area (MPOC) of the Rat Hypothalamus, Journal of Neuroscience Methods, 104:113-121. Accepted: 9/7/2000 **(E0212215)**.

Patterson, T.A., Binienda, Z.K., Newport, G.D., Lipe, G.W., Gillam, M.P., Slikker, W. and Sandberg, J.A., Transplacental Pharmacokinetics and Fetal Distribution of 2',3'-Didehydro-2',3'-Dideoxythymidine (d4T) and Its Metabolites in Late-Term Rhesus Macaques, Teratology, 62:93-99. Accepted: 2/10/2000 **(E0250101)**.

Patterson, T.A., Schmued, L.C., Sandberg, J.A. and Slikker, W., Temporal Development of 2',3'-Dideoxyinosine (ddI)-Induced Peripheral Myelinopathy, Neurotoxicology and Teratology, 22 (3):429-434. Accepted: 12/19/1999 **(E0250201)**.

Paule, M.G., Gillam, M.P., Allen, R.R. and Chelonis, J.J., Effects of Chronic *In-Utero* Exposure to Cocaine on Behavioral Adaptability in Rhesus Monkey Offspring When Examined in Adulthood, Annals of the New York Academy of Science, 914:412-417. Accepted: 7/11/2000 **(E0663300)**.

Paule, M.G., Rowland, A.S., Ferguson, S.A., Chelonis, J.J., Tannock, R., Swanson, J.M. and Castellanos, X., Symposium Overview: Attention Deficit/Hyperactivity Disorder (ADHD): Characteristics, Interventions and Models, Neurotoxicology and Teratology, 22(5):631-651. Accepted: 5/22/2000 **(E0703101)**.

Popke, J., Allen, S.R. and Paule, M.G., Effects of Acute Ethanol on Indices of Cognitive-Behavioral Performance in Rats, Alcohol, 20:187-192. Accepted: 10/1/1999 **(E0691401)**.

Popke, J., Fogle, C.M. and Paule, M.G., Ethanol Enhances Nicotine's Effects on DRL Performance in Rats, Pharmacology Biochemistry and Behavior, 66(4):819-826. Accepted: 3/2/2000 **(E0691401)**.

Popke, J., Mayorga, A.J., Fogle, C.M. and Paule, M.G., Comparison of Drug Effects on the Performance of Two Timing Tasks in Rats, Pharmacology Biochemistry and Behavior, 67:377-385. Accepted: 7/6/2000 **(E0691401)**.

Scallet, A.C., Pothuluri, N.J., Rountree, R.L. and Matthews, J.C., Quantitating Silver-Stained Neurodegeneration: The Neurotoxicity of Trimethyltin (Tmt) in Aged Rats, Journal of Neuroscience Methods, 98:69-76. Accepted: 2/22/2000 **(E0672700)**.

Schmued, L.C., Hopkins, K.J., Fluoro-Jade B: The Next Generation High Affinity Fluorescent Marker of Neuronal Degeneration, Brain Research, 874:123-130. Accepted: 5/16/2000 **(E0701301)**.

Slikker, W., Beck, B.D., Cory-Slechta, D., Paule, M.G., Anger, W.K. and Bellinger, D., Cognitive Tests: Interpretation for Neurotoxicity? (Workshop Summary), Toxicological Sciences, 58:222-234. Accepted: 9/5/2000 **(NA)**.

Slikker, W., Olivero, O., Patterson, T.A. and Poirier, M.C., Potential Toxicities of HIV Therapeutics in the Developing Infant, Teratology, 61:397-398. Accepted: 2/15/2000 **(E0250001)**.

Slikker, W. and Paule, M.G., Cognitive Tests: Interpretation for Neurotoxicity?, World Automation Congress. Accepted: 6/11/2000 **(NA)**.

Slotkin, T.A., Cada, A.M., Ferguson, S.A., McCook, E.C. and Seidler, F.J., Neonatal Polyamine Depletion by α-Difluoromethylornithine: Effects of Adenyl Cyclase Cell Signaling Are Separable From Effects on Brain Region Growth, Brain Research, 887:16-22. Accepted: 9/11/2000 **(E0704001)**.

Walker, L.M., Walker, P.D., York, J., Imam, S.Z., Ali, S.F. and Mayeux, P.R., Evidence for Peroxynitrite Formation in Renal Ischemia-Reperfusion Injury: Studies With the Inducible Nitric Oxide Synthase Inhibitor L-N6-(1-Monoethyl)-Lysine, Journal of Pharmacology and Experimental Therapeutics, 295:417-422. Accepted: 6/1/2000 **(NA)**.

Wang, G., Schmued, L.C., Andrews, A.M., Scallet, A.C., Slikker, W. and Binienda, Z.K., Systemic Administration of Domoic Acid Induced Spinal Cord Lesions in Neonatal Rats, The Journal of Spinal Cord Medicine, 23:31-39. Accepted: 1/21/2000 **(E0692331)**.

Williams, C.J., Soliman, M.I., Duhart, H.M., Slikker, W. and Ali, S.F., Methylcyclopentadienyl Manganese Tricarbonyl-Induced Reactive Oxygen Species in Different Regions of Rat Brain: An *In Vitro* Study, Pharmacology and Neurotoxicology, 5(1and2):23-26. Accepted: 5/30/2000 **(E0671900)**.

Xu, Z., Chang, L.W., Slikker, W., Ali, S.F., Rountree, R.L. and Scallet, A.C., A Dose-Response Study of Ibogaine-Induced Neuropathology in the Rat Cerebellum, Toxicological Sciences, 57:95-102. Accepted: 5/18/2000 **(E0698301)**.

Young, J.F., Gough, B.J., Suber, R.L. and Gaylor, D.W., Correlation of Blood Cholinesterase Levels With Toxicity of Sarin in Rats, Journal of Toxicology and Environmental Health, Part A, 62(3):161-174. Accepted: 8/4/2000 **(E0624700)**.

Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

VETERINARY SERVICES

Director: ***William M. Witt, D.V.M. Ph.D.***
Telephone: **870-543-7949**
Toll Free: **800-638-3321**
E-mail: **wwitt@nctr.fda.gov**

Immunohistochemical procedures in support of Pathology Services provided at the NCTR

Executive Summary

The Division of Veterinary Services (DVS) provides professional and technical support to the various NCTR research Divisions in their efforts to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs.

The Agency's mission, pure and simple, is to protect and promote the nation's public health. Animal-related studies, as those being conducted by the NCTR research community, greatly enhance the Agency's ability to meet this public health mission. The Division has the facilities, equipment and personnel to support this vital interdisciplinary research.

The Division provides administration for the Center's Animal Care and Use Program which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). Included within the Division are the contracted services for animal care, diet preparation, and pathology, all of which are staffed by on-site contract employees.

The "gold standard" for laboratory animal care and use programs is accreditation by the AAALAC International. Such accreditation is widely accepted by the scientific community and indicates that the accredited organization conforms with all government policies and regulations, and that it endorses the highest quality care for the animals involved in their animal use activities. DVS personnel, working through the FDA Research Animal Council (FRAC), have assisted, and will continue to assist, FDA centers in obtaining and maintaining accreditation of their animal care and use programs by AAALAC International.

The Division provides oversight and veterinary management of all laboratory animals and housing facilities at NCTR. Division personnel completed and submitted annual reports assuring compliance with Federal regulations and National Institute of Health (NIH) guidelines relative to the Animal Care and Use Program. Personnel participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR Institutional Animal Care and Use Committee proceedings. The DVS director serves as a member of the FDA Research Animal Committee and its AAALAC International accreditation subcommittee which performs "mock" AAALAC site visits of the various FDA centers prior to actual site visits by AAALAC representatives. Division

personnel serve as government project officers for the pathology services, animal care and diet preparation, and rodent bedding contracts. The Division is responsible for breeding, rearing, and/or acquiring all experimental animals used on-site. A rodent surgical suite is available for performing rodent ovariectomies in support of multigeneration toxicology studies. The Division's personnel also serve as instructors in the on-site technician certification program, which is authorized by the American Association for Laboratory Animal Science (AALAS).

Animal Care/Diet Preparation Services

During 2000, five contract animal care personnel attained certification by AALAS. The average number of experiments supported per month by contract animal care personnel was 75. These experiments entailed as a minimum, the daily animal care support of an average of 6,211 rodents, 16 rabbits, and 118 rhesus monkeys. Technical manipulations for these studies included one or more of the following procedures: tattooing, vaginal lavages, tumor palpations, injections, oral gavage, behavioral testing, and blood collection. Contract diet preparation personnel provided consultation and nutritional support and diet preparation for several carcinogenicity studies including chloral hydrate, malachite green and leucomalachite green, and urethane, funded through the Interagency Agreement with the National Institute for Environmental Health Sciences' National Toxicology Program. During 2000, diet preparation personnel produced dosed diet, autoclaved rodent diet, dosed water, and sized dietary pellets for rodents. Quality Assurance personnel performed quality control audits of contractor-performed procedures.

Pathology and Pathology-Related Services

During 2000, six trainees completed the Laboratory Technician apprenticeship training program and five will take the histotechnician registry examination in April 2001. Personnel worked to develop the use of the Microsoft Access to review, organize and summarize pathology data. Data slides were prepared for pathology personnel and other NCTR researchers using Microsoft PowerPoint and a Polaroid ProPalette 7000 Film Recorder with developing being accomplished with a Jobo Autolab Automatic 35mm processor. Microsoft Project is used to monitor the progress of studies. Contract personnel initiated the use of plastic bottles for long-term storage of wet tissues. An agreement was reached with Southeast Arkansas College for a histology technician training and certification program.

The Pathology staff supported various NCTR research Divisions by providing glutamate synthase assays, *in situ* hybridization support, various ELISA assays, and PCNA assays for the endocrine disruptor studies. In addition, the staff perfected a modified Davidson's fixative for the endocrine disruptor studies and developed immunohistochemical assays in support of the phototoxicity studies.

During 2000, the contract employees authored or co-authored 11 publications or presentations.

FY 2000 Publications

Chou, M.W., Mikhailova, M.V., Nichols, J.A., Poirier, L.A., Warbritton, A.R. and Beland, F.A., Interactive Effects of Methyl-deficiency and Dietary Restriction on Liver Cell Proliferation and Telomerase Activity in Fischer 344 Rats Treated with Aflatoxin B_1, Cancer Letters, 152 (1):53-61. Accepted: 12/1/1999 **(E0695201)**.

Culp, S.J., Warbritton, A.R., Smith, B.A., Li, E. and Beland, F.A., DNA Adduct Measurements, Cell Proliferation, and Tumor Mutation Induction in Relation to Tumor Formation in $B_6C_3F_1$ Mice Fed Coal Tar or Benzo[a]pyrene, Carcinogenesis, 21:1433-1440. Accepted: 3/29/2000 **(E0672202)**.

Dalu, A.N., Blaydes, B.J., Lomax, L.G. and Delclos, K.B., A Comparison of the Inflammatory Response to a Polydimethylsiloxane Implant in Male and Female Balb/c Mice, Biomaterials, 21:1947-1957. Accepted: 3/13/2000 **(E0696301)**.

Hansen, D.K., Laborde, J.B., Wall, K.S., Hinson, W.G., Pipkin, J.L., Shaddock, J.G., Lyn-Cook, L.E. and Young, J.F., Dose-Response of Retinoic Acid-Induced Stress Protein Synthesis and Teratogenesis in Mice, Reproductive Toxicology, 15(1):31-41. Accepted: 9/22/2000 **(E0697601)**.

Howard, P., Eppley, R., Stack, M.E., Warbritton, A.R., Voss, K.A., Lorentzen, R., Kovatch, R. and Bucci, T.J., Fumonisin B_1 Carcinogenicity in a Two-year Feeding Study Using F344 Rats and $B_6C_3F_1$ Mice, Environmental Health Perspectives. Accepted: 8/16/2000 **(E0210601, E0210801)**.

Howard, P., Eppley, R., Warbritton, A.R., Voss, K.A., Lorentzen, R., Kovatch, R.M. and Bucci, T.J., Carcinogenicity of Fumonisin B_1 in a Two-year Bioassay with Fischer 344 Rats and $B_6C_3F_1$ Mice, Mycotoxins (Japan) Meeting Proceedings, S:45-54. Accepted: 10/15/1999 **(E0210601)**.

Howard, P., Warbritton, A.R., Voss, K.A., Lorentzen, R., Thurman, J.D., Kovatch, R. and Bucci, T.J., Compensatory Regeneration as a Mechanism for Renal Tubule Carcinogenesis of Fumonisin B_1 in the F344/N/NCTR BR Rat, Environmental Health Perspectives. Accepted: 8/16/2000 **(E0210601, E0210801)**.

Johnson, J.R., Robinson, B.L., Ali, S.F. and Binienda, Z.K., Dopamine Toxicity Following Long-term Exposure to Low Doses of 3-nitropropionic Acid in Rats, Toxicology Letters, 116:113-118. Accepted: 4/26/2000 **(E0701001)**.

Meredith, J.M., Bennett, C.A. and Scallet, A.C., A Practical Three-dimensional Reconstruction Method to Measure the Volume of the Sexually-Dimorphic Central Nucleus of the Medial Preoptic Area (MPOC) of the Rat Hypothalamus, Journal of Neuroscience Methods, 104:113-121. Accepted: 9/7/2000 **(E0212215)**.

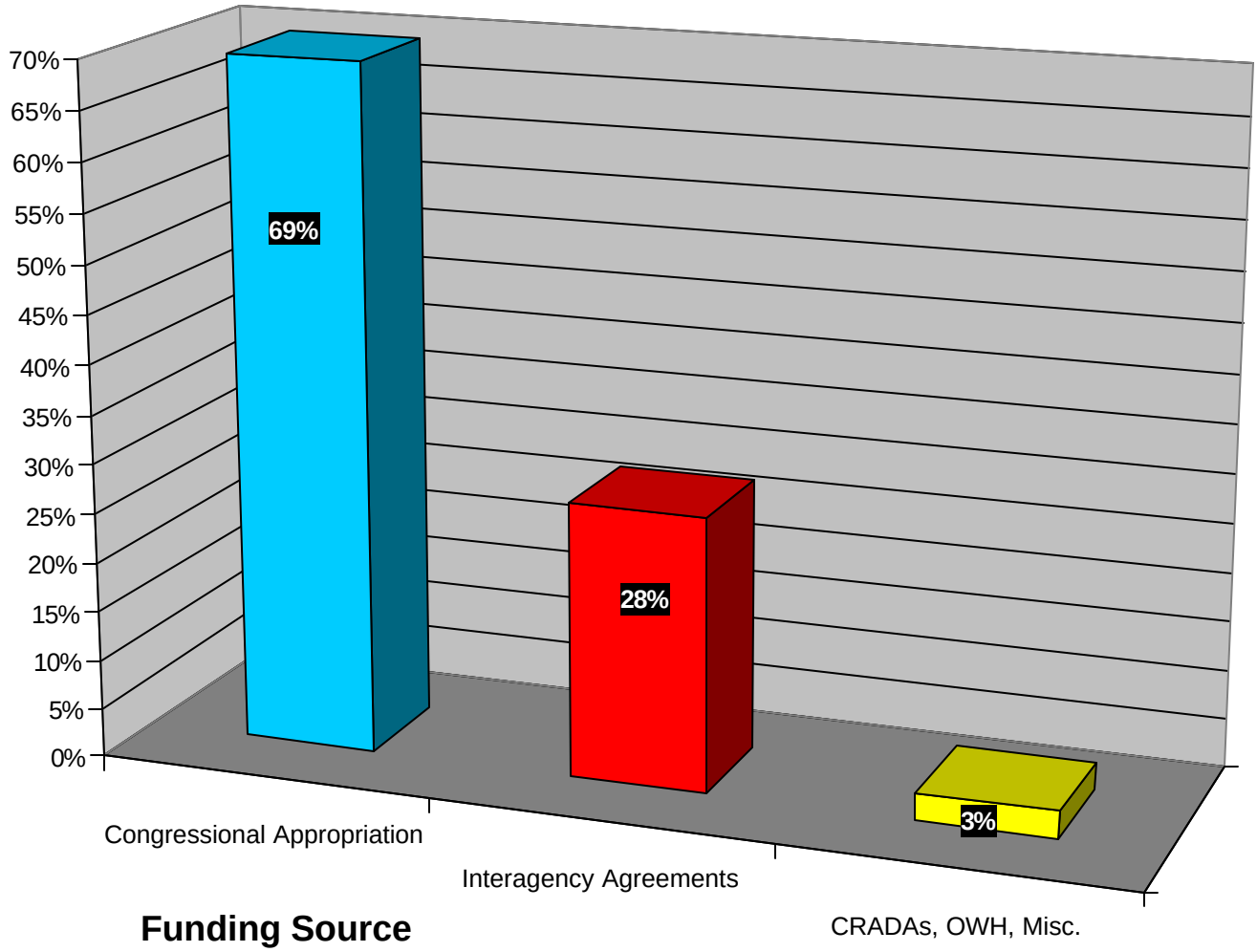
Project Number Codes:
E–Ongoing P–Preliminary S–Support Z-Administrative NA-Not Applicable

Sams, R.L., Blaydes, B.J., Warbritton, A.R., Lomax, L.G., Bucci, T.J., Muskhelishvili, L. and Delclos, K.B., Differences in the Response to Oxidative Stress and Mutation Frequency in CD (Sprague-Dawley) and Fischer 344 Rats Due to an Induced Inflammatory Response, Environmental and Molecular Mutagenesis, 35 (4):336-342. Accepted: 10/23/1999 **(E0699301)**.

Wang, G., Schmued, L.C., Andrews, A.M., Scallet, A.C., Slikker, W. and Binienda, Z.K., Systemic Administration of Domoic Acid-Induced Spinal Cord Lesions in Neonatal Rats, The Journal of Spinal Cord Medicine, 23:31-39. Accepted: 1/21/2000 **(E0692331)**.

SUMMARY OF EXTERNALLY FUNDED PROJECTS*

RELATIVE PROPORTIONS OF NCTR BUDGET



* **Details of projects presented under individual research division reports.**

INTERAGENCY AGREEMENTS (IAGS)

NCTR has been fortunate in establishing Interagency Agreements (IAGs) with other government agencies to conduct research on problems of common interest to the FDA and the collaborating agency. The most significant, in terms of size, is the IAG between FDA/NCTR and the National Institute of Environmental Health Sciences (NIEHS).

With financial support from the National Toxicology Program (NTP), which is conducted under the auspices of the NIEHS, the NCTR has agreed to conduct animal bioassays, mechanistic studies, and risk assessments on a number of compounds of regulatory interest to both the NIEHS and the FDA. This IAG has allowed NCTR to conduct the studies including: mycotoxin fumonisin B_1, a study nominated by the FDA Center for Food Safety and Applied Nutrition (CFSAN); the pediatric sedative chloral hydrate, nominated by FDA's Center for Drug Evaluation and Research (CDER); malachite green, a therapeutic agent used in aquaculture, nominated by FDA's Center for Veterinary Medicine (CVM); and the interaction of ethanol and urethane, nominated by CFSAN. Also, a mechanistic study on riddelliine, a compound of interest to CFSAN, is being supported by the FDA/NIEHS IAG. Studies are also beginning on the risk associated with *Aloe vera* exposure in dietary supplements.

Additional research funded via the FDA/NIEHS IAG includes a series of studies on several endocrine-active compounds including genistein, ethinyl estradiol, and nonylphenol. The studies will determine the endocrine-disrupting effects of these compounds on reproduction, behavior, and carcinogenesis over multiple generations.

As a result of CFSAN's concern about the potential interaction of ultraviolet (UV) light and over-the-counter cosmetics containing alpha-hydroxy acids, support for development of a unique Phototoxicity Research and Testing Laboratory at the NCTR was received from NIEHS/NTP. Risk assessments on a number of FDA-regulated products suspected of interaction with sunlight or fluorescent tube-generated light began in FY 1999.

The Environmental Protection Agency (EPA) has supported NCTR in conducting a broad area of research on neurotoxicity risk assessment, risk assessment associated with waterborne and foodborne pathogens, and support for the development of an endocrine disruptor computerized knowledge base.

As an offshoot of a patent and licensing agreement with Cox Recorders dealing with the detection of decomposed food, The Federal Aviation Administration (FAA) has entered into an agreement with scientists at the Center to explore methods of detecting explosives in airline baggage.

The National Institutes of Health (NIH) and the National Cancer Institute (NCI) are supporting studies at the NCTR into Agent Orange exposure and the mechanism of colorectal cancer, respectively.

Although not an IAG in the strict sense, NCTR has received generous support from the FDA's Office of Women's Health (OWH) for a number of research programs. These include: 1) the investigation of whether a newly developed transgenic animal is an appropriate model for systemic lupus erythematosus; 2) the development of methodologies to assay hydroxylation of endogenous estrogens as that process relates to the risk of developing breast cancer; and 3) research on the effects of dietary supplements on women's health issues.

NCTR has received support from both the FDA's Office of Women's Health and the U.S. Department of Defense (DOD) to conduct molecular epidemiology studies designed to determine the variability in metabolic phenotype and genotype in women with respect to their recurrence of breast cancer following high-dose radiation and chemotherapy.

COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENTS (CRADAS)

In collaboration with Cox Recorders of Belmont, NC, NCTR scientists developed a consumer-based, low-tech indicator of food freshness patented as FreshTag™. Cox Recorders is providing the means of producing one million tags per day and is assisting FDA in getting the product used in the food market more rapidly. This invention was selected as one of the "Best of What's New in 1999" by *Popular Science.*

NCTR's Division of Chemistry received financial support from Scientific Instruments Services, Ringola, NJ, to develop a universal interface for mass spectrometry analysis of high pressure liquid chromatography (HPLC) streams in the analysis of chemicals and metabolites.

NCTR has received support via a CRADA with Genometrix® to develop a "Risk-Tox DNA micro-array" for rapid, high-throughput genotyping. The results of this CRADA will provide FDA the ability to genotype patients for all the major enzyme variants that would predict susceptibility to carcinogens, adverse drug reactions, chemotherapeutic drug efficacy, and individualized dosing of therapeutics.

Both the American Chemistry Council (ACC), formerly Chemical Manufacturers Association (CMA), and the Environmental Protection Agency (EPA) have provided NCTR support for the development of a computerized predictive Estrogen Knowledge Base (EKB). Using Quantitative Structure-Activity Relationships (QSAR), the EKB will be able to screen chemical structures for estrogen activity. The EKB will also serve as a prototype for predicting activity of chemical classes for other activities such as androgens, thyroid hormones and may be applied to other toxic endpoints such as neurotoxicity and carcinogenesis.

NCTR's Division of Neurotoxicology has received financial support from AstraZeneca to study the effects of long-term blockage of glutamate receptors and/or sodium channel blockage on neurobehavioral endpoints in the non-human primate.

UNIVERSITY INTERACTIONS

Many NCTR scientists hold adjunct faculty positions and collaborate with individuals and departments of universities. This practice has been instrumental in leveraging both the intellectual and infrastructure capabilities of NCTR. NCTR scientists have developed research collaborations with more than 20 universities and many scientists have been granted adjunct academic positions. This arrangement permits NCTR staff to develop close collaborative efforts with various university staff to solve problems of mutual interest to FDA and the respective university. Academic collaborations include mutual use of specialized equipment, sharing of research samples to maximize the gain of information from a project, and the exchange of staff between the institutions for lectures, seminars, and conduct of research.

Of particular importance is the close collaborations between NCTR and the University of Arkansas for Medical Sciences (UAMS) in Little Rock, AR. In addition to the adjunct positions held by NCTR scientists at the UAMS, NCTR participates in the UAMS Interdisciplinary Toxicology Program through which graduate students receive a Ph.D. in toxicology. Many of the graduate students perform research for their dissertations in an NCTR laboratory under NCTR staff supervision.

Another example of leveraging with local institutions is that NCTR staff in the Division of Neurotoxicology have access to a behavioral testing laboratory at the Arkansas Children's Hospital (ACH) and at the University of Arkansas at Little Rock, where results of behavioral studies obtained in animals at NCTR are verified in humans at ACH.

Collaborations by NCTR scientists with universities in the U.S. and abroad have resulted in, at no cost to FDA, a number of visiting scientists who come to NCTR to pursue research in areas developed by NCTR scientists. In FY 2000-2001, NCTR hosted more than 46 visiting scientists from the U.S. and 16 foreign countries. These visiting scientists not only contribute valuable scientific expertise to NCTR research programs, but many return to their respective institutions to continue research on problems of interest to FDA and NCTR.

INDEX OF CHEMICALS

a

b

F

1

2

3

4

5

6

7

8

9

17

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

Z

INDEX OF PRINCIPAL INVESTIGATORS

A

B

C

D

E

F

G

H

J

K

L

M

N

P

R

S

T

V

W

X

Y

Z

GLOSSARY OF ACRONYMS AND ABBREVIATIONS

This glossary is provided to assist you in interpreting acronyms, abbreviations, and phrases you encounter while reading this publication. This is not meant to take the place of standard language or Scientific dictionaries, which should be referred to if any short form of scientific terms do not appear in this glossary. Also, you may refer to the Chemical Index, located at the end of this publication as a quick reference to locate other occurrences of a specific term.

4-ABP	4-aminobiphenyl
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AACR	American Association for Cancer Research
AALAS	American Association for Laboratory Animal Science
ACC	American Chemistry Council (ACC), formerly Chemical Manufacturers Association (CMA)
ACH	Arkansas Children's Hospital
ADHD	Attention Deficit Hyperactivity Disorder
ADP	Automatic Data Processing
AIN	American Institute of Nutrition
AP-1	Apurinic-1
APAP	Acetaminophen
APCI/MS	atomospheric pressure chemical ionization/mass spectrometry
AR	androgen receptor
Ars	accumulation rates
ASMS	American Society of Mass Spectrometry
BD IX	rat strain
BF	body fat
BSE	Bovine Spongiform Encephalopathy
CD	Sprague-Dawley
CD	cadaverine
CDER	Center for Drug Evaluation and Research
CE	competitive exclusion
CFSAN	Center for Food Safety and Applied Nutrition
CG	cytosine guanine
CHO	Chinese hamster ovary
CIMA	Computational Intelligence, Methods and Applications
CNS	central nervous system

Co-PI	Co-Principal Investigator
C_pG	cytosine-phosphate-guanine
CR	caloric restriction
CRADA	Cooperative Research and Development Agreement
CRIMS	chemical reaction interface mass spectrometry
CVM	Center for Veterinary Medicine
DdC	2',3'-dideoxycytidine
DD-PCR	differential display PCR
DHR	dehydroretronecine
DMN	dimethylnitrosamine
DNA	deoxyribonucleic acid
DOD	Department of Defense
DR	dietary restriction
ECD	electrochemical detection
EDKB	Endocrine Disruptor Knowledge Base
EE2	ethinyl estradiol
EEG	electroencephalogram
ENAR	Eastern North American Region
EPA	Environmental Protection Agency
ER	estrogen receptor
FAA	Federal Aviation Administration
FDA	Food and Drug Administration
FFM	fat free mass
FISH	florescent NC2 hybridization
FRAC	FDA Research Animal Council
FT	fourier transform
FY	fiscal year
GAT	guanine adenine thymidine
GB	ginkgo biloba
GD	gestational day
GGT	guanine guanine thymidine
GLP	Good Laboratory Practice
GS	goldenseal
GSA	genotypic selection assay
GST	glutathione S-transferase
GSTP1	glutathione S-transferase P1

GTT	guanine thymidine thymidine
HaCaT	keratinocyte cell line
HGSTAl	human glutathione S-transferase Al
hGSTM1	human glutathione S-transferase M1
hGSTT1	human glutathione S-transferase T1
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HPLC/EC	high performance liquid chromatography/EC
HS	histamine
IA/LC/MS	Immuno affinity/liquid chromatography/mass spectrometry
IAG	interagency agreement
IAOAC	International Associatioon of Official Analytical Chemists
IARC	International Agency for Cancer Research
IC-50	inhibition curve-50
ICSA	International Chinese Statistical Association
IGF-1	insulin growth factor-1
IgG	immune sera
ILSI	International Life Sciences Institute
LC	liquid chromatography
LC/MS	liquid chromatography-mass spectrometry
LC/PDA	liquid chromatography/photo diode array
LC-APCI/MS	liquid chromatography-atmospheric pressure chemical ionization mass spectrometry
LC-ESI/MS	liquid chromatography-electron spray ionization/mass sprectroscopy
(MAB)/MS	metastable atom bombardment/mass spectrometry
MALD FT	matrix assisted laser desorption fourier transformed
MALDI/MS	matrix assisted laser desorption-mass spectrometry
MALDI/TOF-MS	matrix assisted laser desorption/time of flight-mass spectrometry
MALDI-TOF MS	matrix assisted laser desorption ionization time-of-flight mass spectrometry
MD	methyl deficiency
MFs	mutant frequencies
MHz	megahertz
MRNA	messenger RNA
MS	mass spectrometry
MS	methionine synthase

NA	not applicable
NCCRI	National Cancer Center Research Institute
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
NIA	National Institute on Aging
NIEHS	National Institute of Environmental Health Sciences
NMR	nuclear magnetic resonance
NOAA	National Oceanic and Atmospheric Administration
N-OH-IQ	N-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline
NONMEN	nonlinear mixed-effects modelling
NOS	nitric oxide synthase
NTF	neurogrowth/neurotrophic factor
NTP	National Toxicology Program
Oltipraz	5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione
ORA	Office of Regulatory Affairs
ORISE	Oak Ridge Institute for Science and Education
OTB	operant test battery
OTC	oxytetracycline
OWH	Office of Women's Health
PAGE	polyacrylamide gel electrophoresis
PAH	polycyclic aromatic hydrocarbon
PattRec	pattern recognition
PBPK	physiologically-based pharmacokinetic
PC-12	cell culture
PCR	Project on Caloric Restriction
PCR	polymerase chain reaction
PFGE	pulse field gel electrophoresis
PhIP	2-amino-1-methyl-6-phynelimidazo[4,5-f]pyridine
PI	Principal Investigator
ppb	parts per billion
PyMS	pyrolysis mass spectrometry
QSAR	quantitative structure-activity relationships
RBA	relative binding affinity
RFD	rank fish detector
Romet-30	sulfadimethoxine-ormetoprim
ROS	reactive oxygen species

RT-PCR	Reverse Transcriptase – polymerase chain reaction
SA	sulfonamide
SAB	Science Advisory Board
SAR-SDAR	Structure Activity Relationship-Spectral Data-Activity Relationships
SCR	sample collection report
SD	Sprague Dawley
SDARs	spectral data-activity relationships
SDH	succinate dehydrogenase
SDN	sexually dimorphic hypothalmic nuclei
SFC	supercritical fluid chromatography
SHR	spontaneously hypertensive rat
SNARC	Stuttgart National Aquaculture Research Center
SNP	single nucleotide polymorphism
SOT	Society of Toxicology
SSCP	single-strand conformation polymorphism
SUNY-SB	State University of New York at Stony Brook
TBW	total body water
TCR	transcription coupled repair
TGE	total gestational exposure
TLC	thin layer chromatography
TOBEC	total body electrical conductivity
TSNAs	tobacco specific N-nitrosamines
TVB	total volatile bases
UALR	University of Arkansas at Little Rock
UAMS	University of Arkansas for Medical Sciences
Urethane	ethyl carbamate
USDA	United States Department of Agriculture
UV	ultraviolet
UVB	ultraviolet (B indicates the region)